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PROGRESS

ALLERGY OF THE ABDOMINAL ORGANS*

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ALTHOUGH it has long been recognized that abdominal organs may participate in allergic reactions, there have been relatively few reports dealing with this subject. Because of the inaccessibility of the abdominal organs, considerable speculation has arisen as to the seat of the allergic processes and the nature of these reactions.

My colleagues H. Straus, I. Gray, M. Harten, S. Livingston, and D. Grayzel, and I have attempted to reproduce and study allergic reactions of the abdominal organs under experimental conditions. Investigations, not feasible in human beings, were carried out in the rhesus monkey, in which it is possible to induce allergic reactions closely resembling those occurring in man. It is hoped that the brief analysis of these experimental findings presented herewith will facilitate a better understanding of allergic reactions occurring within the human abdomen.

The primary experimental principle applied in most of these investigations was the production of allergic reactions in tissues which had been passively sensitized locally with human serum, containing atopic reagin antibodies. These sera were obtained from allergic individuals who were clinically very sensitive to such allergens as cottonseed, peanut, fish, egg, etc., and who showed very strong cutaneous reactions when tested with weak extracts of these allergens. This cutaneous sensitivity could be transferred to the skin of normal individuals by an intracutaneous injection of 0.05 ml. of the reagin-containing serum. The allergic reaction at the sensitized site was elicited from twenty-four to forty-eight hours later by feeding the related antigen, i.e., peanut, cottonseed, fish, egg, etc., or by an intravenous injection of an extract of that antigen. Oral administration of the antigen was regularly followed, within from five to

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fifteen minutes, by the entrance of traces of the antigen into the circulation and by the formation of a wheal at the sensitized cutaneous site.[1] This reaction resulted from the union of the circulating antigen with the reagins, which had remained fixed in the skin at the site of sensitization. The intravenous injection of the antigen usually resulted in a more rapid onset and in a more intense reaction than that which occurred following enteral administration. Both methods of inducing the allergic reaction were employed, depending upon the needs of the experiment. In the earlier investigations sensitization was confined to the skin; in later studies, mucous membranes, other tissues, and even complete organs were successfully sensitized.

METHODS OF EXCITING ALLERGIC REACTIONS IN ABDOMINAL ORGANS

Under physiologic conditions offending allergens may reach and excite abdominal organs in two ways. Any specific excitant introduced into the alimentary tract may, by direct contact, induce allergic reactions in the organs through which it passes. It is also possible for any antigen which gains entrance into the blood stream to reach sensitive abdominal organs by this route. The most natural way for antigens to reach the circulation is by way of the alimentary tract. In our studies on normal individuals, fish, egg, nut, cottonseed, mustard, milk, and other antigens almost routinely appeared in the blood stream within from two to thirty minutes after their ingestion.[1] This rapid absorption of the allergen accounts for many of the explosive allergic reactions which have been seen to follow, almost immediately, the ingestion of offending allergens. These reactions may affect widely separated organs, including parts of the digestive system distant from the site of administration and absorption of the excitant. That any one of several closely related organs or only one type of tissue may participate in an allergic reaction, while others in the same individual appear to remain unaffected, is recognized as a common occurrence in allergy, although an adequate explanation for this phenomenon is still lacking.

Passage of allergens into the circulation has been demonstrated to occur almost regularly from all parts of the alimentary tract, including the esophagus, stomach,[2] duodenum,[3] ileum, colon,[4] and rectum.[5] Gastric acidity is an important factor in influencing the amount and rapidity of allergenic absorption. Gastric hyperacidity definitely retards and diminishes absorption, not only from the stomach and duodenum but also from the rectum.[5] Hypoacidity and anacidity produce the opposite effects. Absorption of allergens may also be delayed or prevented by the previous oral administration of other food, oil, kaolin, peptone, hydrochloric acid, or similar substances.[6] Alcohol promotes and accelerates absorption. The importance of these experimental findings in the management of food allergies is obvious.

Allergen, administered rectally, may enter the blood stream as rapidly as that introduced into the duodenum, and even more rapidly than that taken by mouth.[5] It should also be remembered that other mucous membranes and lining surfaces permit the rapid passage of antigens. Hence, sensitive abdominal organs may be specifically excited by allergens which have been absorbed by way of the upper and lower respiratory tract[7, 8] and through the vaginal and cervical

mucous membranes.[9] The same may be said in relation to allergens which have been introduced into the pleural, pericardial, and peritoneal cavities,[10] and into the urinary bladder[10] and the gall bladder.[2] Similar effects may also occur when antigen is rubbed into the skin[11] or administered by the intraspinal,[10] intramuscular, intracutaneous, or subcutaneous routes.

Minute quantities of antigen, containing as little as 0.0001 mg. of nitrogen, when given intravenously, have proved sufficient to induce maximal allergic reactions in passively sensitized tissue.[5] The severity of allergic symptoms may, therefore, be far more intense than might be expected from relatively slight contact with the excitant. Moreover, it seems likely that in allergic incidents involving abdominal as well as other shock organs, observers are apt to overlook the importance of less obvious contacts with excitants, such as those occurring by inhalation and by vaginal, rectal, or parenteral administration.

ALLERGIC REACTION IN PASSIVELY SENSITIZED INTESTINAL MUCOUS MEMBRANE IN HUMAN BEINGS

An insight into the nature of the allergic reaction in the human alimentary mucous membranes was obtained by studies of the experimental allergic reaction induced in the sensitized mucous membrane of the rectum.[12] In this study the antigen was administered either rectally or orally. The reaction at the sensitized mucous membrane site appeared in from five to twenty minutes.

Edema, hyperemia, and hypersecretion were the cardinal characteristics of the reaction developing at the sensitized mucous membrane site. Edema appeared first, associated with a pallor of the mucous membrane which was superseded, within a few minutes, by marked hyperemia. Hypersecretion of mucus occurred over the affected area. The entire reaction reached its peak within fifteen minutes and then began to recede rapidly, disappearing almost completely in about an hour. The subjective symptoms varied with the site of sensitization. Pruritus, rectal fullness, and burning, as well as a desire to empty the bowel, were experienced in variable degree, depending on the site of the reaction. The closer the reaction to the anus, the more varied and intense were the symptoms. Few subjective symptoms were noted when the distance from the anal orifice was as much as 6 cm.

To observe the reactions in the ileum and colon mucous membranes, two patients, one with an ileostomy, the other with an ileocolostomy, were studied.[4] Sites on the exposed mucous membranes were sensitized by intramucosal injections of reagin-bearing serum. Within four or five minutes following oral administration of the antigen, the allergic reaction started. Pallor and hypersecretion of the sensitized area were followed, in a few minutes, by edema which became progressively greater for about half an hour (Fig. 1). The pallor was replaced by marked hyperemia and the secretion of mucus became profuse. The entire reaction receded at the end of an hour. Except for itching or burning of the surrounding skin, no subjective symptoms accompanied these reactions.

Direct application of antigen, on an applicator, to a sensitized mucous membrane site on the rectum[12] or colon,[4] produced an allergic reaction of moderate intensity in about four or five minutes. As a result of this application, sufficient antigen was absorbed to excite a previously sensitized *cutaneous* site. At

this time, the mucous membrane sites began to react maximally. This sequence of events, noted repeatedly in these and other experiments, again emphasizes the importance of blood-borne antigen in the elicitation of maximal allergic responses. Although direct contact of mucous membranes with offending allergens may produce severe reactions, it seems likely that, in most instances, reactions of maximal intensity are caused by blood-borne excitants. It is of interest to note that, in the above experiments, the antigen, which was gently applied on an applicator to the sensitized mucous membrane site (without rubbing), reached the circulation by passing through the sensitized area itself. Sensitive mucous membrane, therefore, is not a barrier to the absorption of offending allergens.

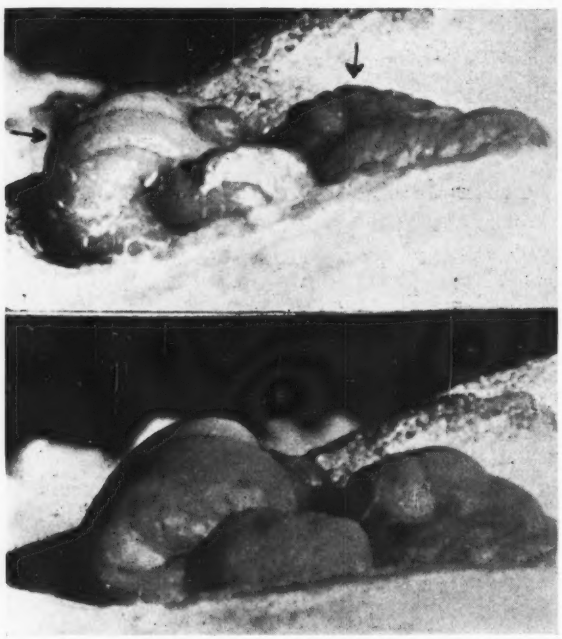

Fig. 1.—*Above,* Human ileum (left) and colon (right) at onset of allergic reaction. *Below,* Human ileum (left) and colon (right) after thirty minutes, at height of allergic reaction. Arrows mark sites of sensitization.

ALLERGIC REACTIONS IN PASSIVELY SENSITIZED ORGANS IN THE MONKEY

In the monkey sensitization of mucous membrane was accomplished with laparotomy under sterile precautions, by the injection of sensitizing serum into mucous membranes exposed by incisions into various organs.[13] One or more sites were sensitized in areas as far away as possible from the neighborhood of the incision into the organ. At times intussusception of one organ into another which had been opened permitted sensitization of the first without actual incision into it. Either the oral or the intravenous method of excitation of the allergic reaction was employed.

In studies on the passively sensitized stomach[13] in the monkey, the most pronounced changes occurred in the uncut organ in which the mucous membrane had been sensitized at several sites, through an opening in the duodenum, near the pylorus. The administration, by stomach tube, of the meal containing the

antigen resulted in the onset of the allergic reaction within three or four minutes. At first the walls of the organ became pale and turgid. Peristaltic waves increased in frequency and intensity. Prepyloric and pyloric spasm developed and persisted for long periods of time. The size of the organ became definitely diminished. On section the mucous membrane was seen to be markedly hyperemic and edematous. This was particularly noticeable at the pylorus, which was almost closed by thickening of the mucous membrane on the gastric side, while the lining of the unsensitized duodenum remained normal. Mucus flowed excessively from the gastric mucous membrane.

Fig. 2.—Opened gall bladder in the monkey showing allergic reaction involving the lower one-half of the organ.

In the cecum and colon, in which single sites of the mucous membranes had been sensitized, similar reactions developed.[13] With the onset of the allergic reaction, blanching of the affected gut developed, followed in a short time by local swelling and edema. Hyperperistalsis became noticeable in this region of the bowel and, in some instances, definite tonic contractions, lasting for five or ten minutes at a time, were noted at the sensitized area. On section of the bowel, the mucous membrane revealed the usual picture of hyperemia, marked edema, and hypersecretion.

Microscopic examination[14] of tissue taken from the site of the allergic reaction showed the lining cells of the mucosa to be only slightly involved. The submucosa in particular, and, to some extent, the serosa were edematous. Their vessels were distended and showed margination of the white blood cells. Around the vessels and throughout the tissues were infiltrations of eosinophiles, lymphocytes, large mononuclear and some polymorphonuclear leucocytes.

In experiments on the gall bladder[15] incision into the organ was avoided. Sensitization was effected by injecting the sensitizing serum directly into the wall of the organ at several sites. The allergic reaction was induced by intravenous injection of the antigen. Within a minute or two that part of the organ which had been sensitized grew pale and the surface vessels became more prominent. This part of the wall became edematous and appeared to bulge and throb. Spasm was not observed in any part of the organ in any of these experiments.

On section of the gall bladder at the height of the reaction the wall of the involved area appeared several times thicker than that of the unaffected part. As in the other organs, edema, hyperemia, hypersecretion, and loss of surface markings characterized the allergic mucous membrane (Fig. 2).

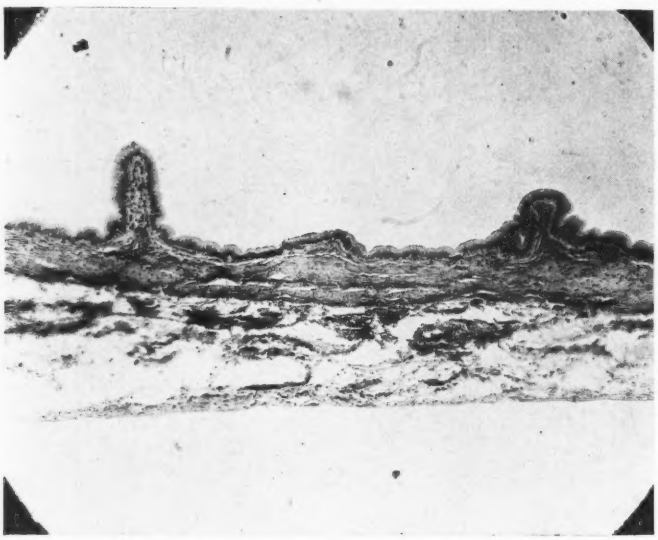

Fig. 3A.—Microscopic picture of normal part of the gall bladder in the monkey.

Microscopic examination of the allergic gall bladder wall revealed the mucosal epithelium to be intact and only slightly affected (Fig. 3). The lamina propria was markedly edematous. The blood vessels were engorged and showed some margination of white blood cells. There was a pronounced cellular infiltration consisting of eosinophiles, lymphocytes, large mononuclear cells, and some polymorphonuclear leucocytes. The edema invaded the other layers of the gall bladder, spreading between the muscle bundles and fibers. Vascular congestion and cellular infiltration also were present in these areas but not to the same degree as in the lamina propria. The nonsensitized part of the organ showed none of these changes on microscopic examination.

ALLERGIC REACTIONS IN THE ALIMENTARY TRACT OF FOOD-SENSITIVE CHILDREN

Special roentgenographic studies in food-sensitive children, made by Fries and Zismor,[16] rendered it possible to visualize, to some extent, the behavior of the human alimentary tract during allergic reactions. Thirty children, who were known to manifest gastrointestinal disturbances following the ingestion of certain foods, were first studied by fluoroscopic and roentgenographic observa-

tions following the ingestion of a standard barium meal. About a week later a similar meal, to which, without the patient's knowledge, a small amount of an offending allergen had been added, was administered under the same experimental conditions. The x-ray findings were then compared with those of the control series.

Some characteristic changes were found in many of these children, following the ingestion of the allergen-barium meal. In most instances a pronounced gastric retention developed. The stomach appeared hypomotile and hypotonic. The duodenal bulb, in many cases, seemed to fill with difficulty. At times barium was still observed in the stomach six hours after the allergen-barium meal, whereas the control series had shown complete emptying of the stomach in a much shorter time.

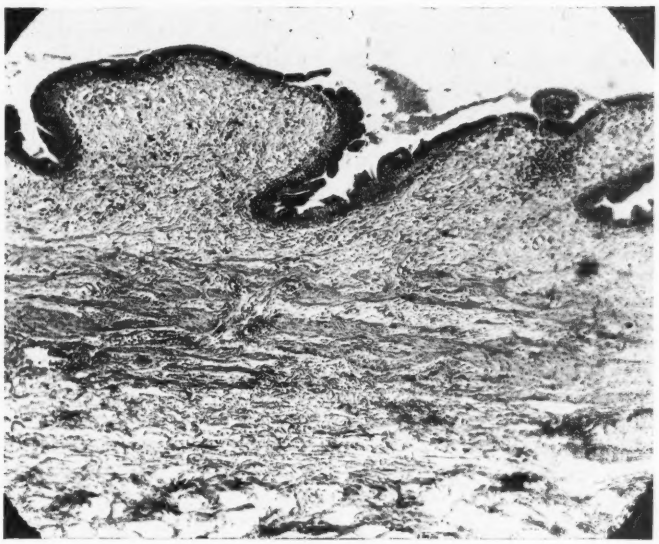

Fig. 3*B*.—Microscopic picture of gall bladder wall involved in allergic reaction.

The progress of the allergen-barium meal through the small bowel varied with the patient. In some its passage was definitely accelerated, while in others no unusual feature was detectable in its progress. In the large bowel evidences of spasm or dilatation were frequently observed. This finding was not constant and, whenever present, it did not affect all portions of the bowel equally.

The administration of allergen-barium enemas to the allergic children usually precipitated spasm in some parts of the large bowel. The seat of the tonic contractions varied with the patient, although the transverse colon seemed to be affected most frequently. In some dilatation of the bowel resulted from this contact with the offending allergen.

The oral administration of the allergen-barium meal in 30 food-sensitive children produced subjective symptoms in 73 per cent.[17] Nausea was noted most frequently, occurring in 43 per cent. Vomiting, usually within several minutes after administration of the meal, occurred in 23 per cent. In 20 per cent abdominal pain was pronounced. This pain had no characteristic localization, but

was most frequently reported to be in the center of the abdomen. Burning or itching of the mouth or throat was a complaint of only 7 per cent.

Abdominal pain was a more frequent and severe symptom among the 16 children who received allergen-barium enemas. Seventy-five per cent of these experienced severe abdominal pain. Nausea occurred in only 6 per cent of this series, but tenesmus was a complaint of 24 per cent.

ALLERGIC SHOCK TISSUE

The foregoing findings suggest apparent similarities in the allergic reactions of the gastrointestinal tracts in man and in the passively sensitized rhesus monkey. In both, edema, hyperemia, and hypersecretion characterize the allergic mucous membranes. In both, the emptying time of the stomach is delayed, following the ingestion of the offending allergen. Spasm of the small and large bowels may occur in both, although it is not so pronounced or so regular a finding in man as it is in the monkey. Dilatation of the bowel was observed more frequently in man.

These observations render it necessary to reopen the question as to whether the smooth muscle is the seat of the allergic reaction in man and plays the same role as shock tissue that it does in the anaphylactic reaction of the guinea pig, rabbit, and dog. This assumption, accepted as fact in some quarters, finds no support in the above findings. Smooth muscle spasm, which characterizes the anaphylactic reaction in the guinea pig, is, by no means, a constant manifestation of the allergic reaction of the human bowel. Moreover, on testing intestinal smooth muscle of the passively sensitized monkey by the Schultz-Dale technique, Albert and I[18] found no evidence of sensitivity of this tissue. Negative results were obtained with these intestinal strips, despite the fact that these animals had been passively sensitized with large doses of human reagin-bearing sera of high titer, which easily produced sensitization of the skin and mucous membrane of the same animals. Even when anaphylactic (precipitin) antibodies were employed for passive sensitization of the rhesus monkey, no evidence of sensitivity could be found in the smooth muscle of intestinal strips.[19] Negative results were also obtained in monkeys in which active sensitization was attempted using the technique suggested by Kopeloff, Davidoff, and Kopeloff.[20]

In man, the only smooth muscle studies reported are those of Tuft,[21] who tested uterine muscle strips obtained during the cesarean section of a woman with precipitins and reagins to horse serum in her blood. Tests with horse serum, by the Schultz-Dale technique, failed to produce any specific contractions in the uterine muscle. It, therefore, seems unlikely that, in man as well as in the monkey, smooth muscle is the shock tissue. This viewpoint finds further support in the histologic studies of the allergic reactions already described. It will be recalled that, although edema, vascular dilatation, and cellular infiltration involved the connective tissue around the muscle bundles and between the muscle fibers, the latter did not seem to share in the inflammatory process.

It also seems unlikely that the epithelial cells of the mucous membranes are the seat of the allergic reactions. These cells did not appear to be seriously altered in the histologic studies of the allergic tissues. Moreover, the

topical application of an allergen to the surface of the sensitized rectal or intestinal mucous membrane did not elicit a maximal allergic reaction, an experimental finding similar to that which had been noted in connection with the passively sensitized nasal mucous membrane.[22] The fact that blood-borne antigen, even though diluted, was able to elicit more intense allergic responses than concentrated antigen applied directly to the epithelial surface tends to minimize the importance of the latter as a shock tissue.

OTHER ABDOMINAL ORGANS AND TISSUES SHARING IN ALLERGIC REACTIONS

In the histologic studies of the allergic abdominal organs, it was repeatedly noted that the peritoneum shared in the inflammatory reactions. When the sensitizing sera were introduced into the serosa of the intestine, the allergic reaction, subsequently elicited, was most pronounced in the peritoneal layer.[13] The edema, in such an instance, tended to surround the gut and to affect the muscular and mucosal layers only to a slight degree. Frequently, the edema spread between the layers of the mesentery, increasing its thickness considerably. An increase of secretion was definitely noticeable over the affected serosa. It was also of interest to note that, when the peritoneum was the seat of the allergic reaction, tonic spasm of the bowel in this region was not commonly observed as it had been when the mucous membranes had been sensitized. All these findings suggest that the peritoneum itself may be the primary seat of an allergic reaction and that allergic peritonitis should be recognized as a possible cause of abdominal disturbances in allergic individuals.

Allergic cholecystitis is another clinical possibility which is not being given sufficient consideration in the differential diagnosis of pain in the right upper quadrant in allergic individuals. Experiments in the monkey, demonstratng that this organ can be sensitized, and the pathology of the allergic reaction occurring in the gall bladder have already been presented.[15] Allergic involvement of the gall bladder may possibly account for the frequent failure to find roentgenographic evidence of gall bladder disease in allergic patients with the typical symptoms of cholecystitis. Moreover, the transient nature of the inflammatory process in allergy may account for the absence of pathology in the gall bladder by the time these patients reach the operating table after a protracted period of observation and preparation. The diagnosis, however, may be established by the fact that the feeding of the specific allergen induces gall bladder symptoms at will, while its elimination from the diet produces a complete cessation of symptoms.

Attempts to demonstrate sensitization of other abdominal organs and tissues have met with only partial success.[13] The allergic reaction in the sensitized spleen in the monkey was a brief one. Following intravenous administration of the offending antigen, the spleen swelled rapidly and assumed a darker color. The process was only short-lived, however, and the organ returned to its normal state within a few minutes.

The allergic reaction in the passively sensitized uterus[13] appeared, for the most part, to involve the peritoneum and subperitoneal connective tissue. The uterine muscle and endometrium seemed hardly to share in the edema.

Injection of the reagin-bearing serum into a lobe of the liver did not induce sensitization of hepatic tissue, and no evidence of an allergic reaction in this organ has thus far been obtained.[13] Attempts at sensitization of renal tissue have invariably resulted in death of the animal before the experiment could be completed.

DISCUSSION

Although the reagin mechanism has been used to reproduce experimentally the allergic reactions in the various abdominal tissues and organs, it is not my intention to suggest that all allergic reactions are mediated by this antibody. It is, in fact, common knowledge that food hypersensitiveness occurs in the absence of reagins and, therefore, of positive cutaneous reactions to the offending allergen. In such instances, the clinician is compelled to rely on clinical trials and diets for the diagnosis. A close scrutiny of the patient's daily food diary and analysis of his symptoms in relation to each article of his diet may be necessary for the detection of the offending allergen. Coca[23] has recently suggested pulse rate studies as a means of diagnosis in nonreaginic food allergies.

Regardless of the absence of reagins in many forms of food hypersensitiveness, their use in the above investigations has yielded some definite experimental information as to the nature of the processes involved in abdominal allergy and opens the way for still further investigation of this little explored field.

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CLINICAL AND EXPERIMENTAL

FAMILIAL NONREAGINIC FOOD ALLERGY*

Its Specific Diagnosis and Treatment

Arthur F. Coca, M.D., Oradell, N. J.

IN TWO previous informal publications[1, 2] it was stated that certain allergic clinical reactions to foods were so constantly accompanied by tachycardia that in all the few cases that had been observed the offending foods could be identified through the use of that objective criterion.

This observation seemed especially welcome because the usual cutaneous tests for food sensitivity failed in nearly all the cases studied—as they have in nearly all the 19 succeeding ones that were so tested.

The findings were reported informally because the cases seemed too few to be acceptable in the usual media of publication, and yet interesting enough to suggest a further trial of the method of diagnosis in suitable cases.

More recently, additional cases have become available for study, and it is now evident that another category of familial allergic disease must be recognized as quite distinct from the previously recognized atopic group of bronchial asthma, hay fever, and atopic dermatitis. Briefly, the separation of the two categories is required on account of (1) differences in symptomatology; (2) difference in mechanism; and (3) difference in the hereditary factor. Only the first two differences will be discussed in the present report. However, the two terms that have been tentatively chosen to designate the two categories will be used for convenience in this paper. These are *atopy* (reaginic asthma, hay fever, atopic dermatitis) and *familial nonreaginic food allergy* (urticaria, allergic headache—migraine, gastrointestinal food allergy, and a number of other more or less serious clinical manifestations).

This investigation has already taken several different directions, detailed mention of all of which cannot be made in a single report of this kind. It is intended, in this first paper, to present evidence to indicate: (1) that specific tachycardia is a reliable criterion of nonreaginic food-allergic reaction; (2) that this criterion can be practically employed with a high percentage of success in the complete relief of food-allergic symptoms.

The eight charts presented are examples of transient tachycardia in some of which there is a close association with the ingestion of a particular food and with one or more clinical symptoms of food allergy.

In these graphic records of the pulse rate in four of the 31 persons who have been relieved of their food-allergic symptoms under the regime of trial diet that

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has been described elsewhere,[1, 2] three characteristic phenomena are illustrated: (1) the rapidly alternating tachycardia and remission of the high pulse rate; (2) the latent period of the food-allergic reaction, and (3) the recurrent reaction.

The patient referred to in Chart 1 is a particularly interesting object of study because she was subject to paternally inherited atopic asthma and hay fever (pollen, house dust, wheat), as well as to the nonreaginic food allergy which she inherited from her mother. Ingestion of wheat, especially postum, causes an attack of asthma but no tachycardia, and her serum passively sensitizes the normal human skin, markedly to house dust and distinctly, though not markedly, to wheat. On the other hand, potato, pork, orange, and coffee, all cause tachycardia, headache, and other clinical symptoms of nonreaginic food allergy (Table I), but the patient's serum does not passively sensitize the normal human skin to these four foods.

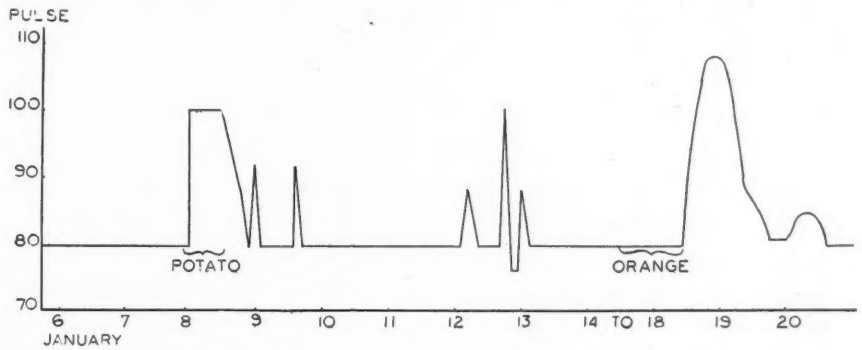

Chart 1.—The course of the pulse rate in patient M. M. D. on the sixth to the twentieth day of observation.

This demonstrated coincidental occurrence of the reaginic atopy and the nonreaginic food allergy in the same person suggests the possibility that the two forms occasionally may be exhibited by the same person to the same food. Hence, it will not be necessary, if, in the blood of a patient with migraine (for example), atopic reagins should be demonstrated that are specific for an excitant of it, to conclude that the reagins in that instance represent the specific mechanism of the migrainous attack.

The following additional features of this case are of interest:

(1) Beef, puffed wheat, or bread, milk, and twice-ultrafiltered lemon juice were taken throughout the period indicated in Chart 1.

(2) Although potato was discontinued after lunch on the eighth day, the pulse rate rose twice on the ninth day. This recurrence of symptoms after discontinuance of an allergenic food is also illustrated in the record of the pulse rate after the ingestion of orange by this patient. There was mild recurrence of tachycardia and also symptoms (headache) on the second day after the discontinuance of orange.

(3) Orange was taken in liberal quantity at all three meals for nearly two days before symptoms appeared. This was the first observed instance in this study of the "latent period," which presently will be discussed more fully.

(4) The causes of the brief but considerable acceleration of the pulse rate on the twelfth day were not discovered. There may have been an accidental addition of some unsuspected allergen in the diet on that day.

(5) The level line at 80 indicates the highest recorded pulse rate (observed before and after each meal) during the indicated period. The pulse rate was frequently lower—sometimes as low as 72.

(6) Tachycardia was sometimes present for an appreciable time after the other symptoms had disappeared. In another person noticeable tachycardia has frequently been absent when the patient was suffering severe heartburn due to ingestion of relatively small quantities of an allergen.

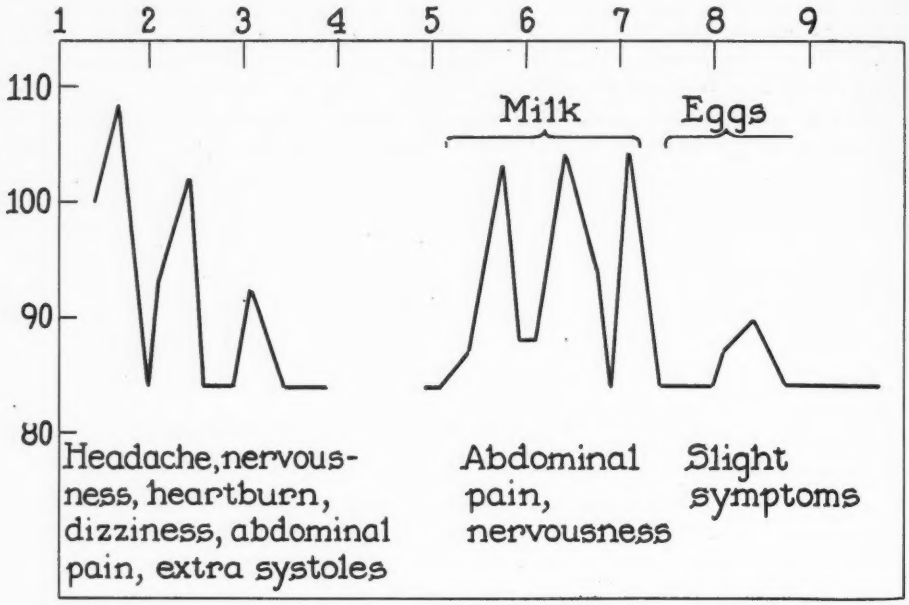

Chart 2.—The course of the pulse rate in patient A. R. on three days previous to the period of trial diet, and on the first five days of the trial diet.

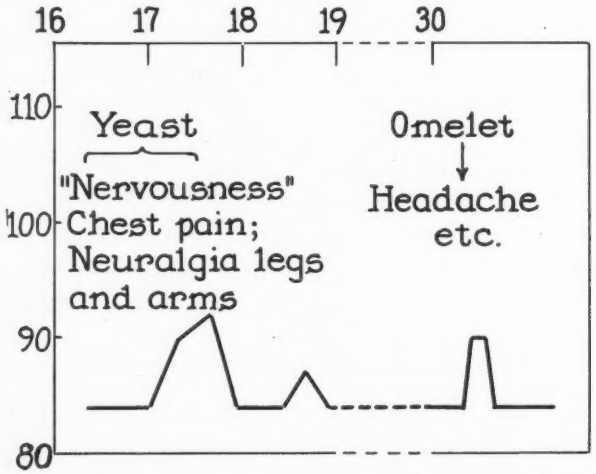

Chart 3.—The course of the pulse rate in patient A. R. on the sixteenth to the thirtieth days of observation.

A young biological chemist, A. R., whose pulse record is illustrated in Charts 2 and 3, had since February, 1939, been suffering from the symptoms mentioned in Chart 2 and had lost 25 pounds in weight. The pulse record shown under the figures 1 to 4, representing the first four days, shows the range of the pulse rate over 84 (which was found later to be his normal maximum) on his usual diet, which included generous amounts of milk and bread. On the fifth and sixth days, and on the morning of the seventh day, he took only milk every hour, and became convinced, at the end of this trial, that milk does not agree with him. He changed to a pure

egg diet from lunch on the seventh day and his symptoms lessened, the pulse rate on the eighth day showing a slight recurrent reaction to milk. After the ninth-day period he added wheat, beet sugar, and pork to his diet without experiencing symptoms, his pulse remaining under 84.

Following the ingestion of a yeast-raised zwieback, his symptoms recurred with a mild tachycardia. On the sixteenth and seventeenth days, he took several cakes of Fleischmann's yeast and, after a latent period of about a half day, experienced clinical symptoms with tachycardia. On the eighteenth day his pulse registered a slight, recurrent reaction. During the period between the nineteenth and thirtieth days, in which other foods were added to the diet, he was symptom free. The thirtieth day he spent in New York City where, as he reported, he "became overexcited and fatigued by climbing subway and elevated steps"; he ascribed to this unusual experience the headache and tachycardia recorded on that day. However, inquiry revealed that at lunch he had eaten omelet, not being aware of the milk content of this dish. He has been symptom free during the succeeding eighteen months and has gained 35 pounds in weight.

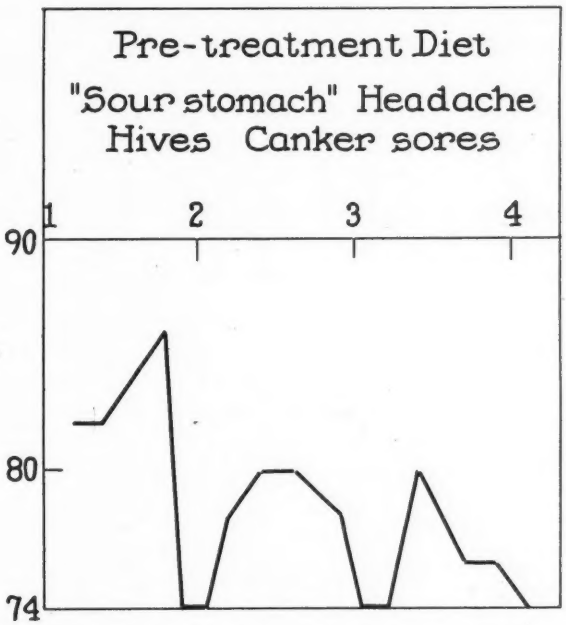

Chart 4.—The course of the pulse rate in patient J. G. on three days prior to the period of trial diet.

Trials with 30 different foods have revealed no other sensitivities.

The serum of this patient does not cause the slightest detectable passive sensitization of the human skin to milk.

J. G., a 36-year-old man, active in the management of a large city garage, complained chiefly of a headache on the left side which recurred at one- to two-week intervals and incapacitated him for about three days. The recurrent headaches were of eighteen years' duration, and the most serious step in his previous treatment of them had been an exploratory abdominal operation performed at his insistence. Charts 4 and 5 show the course of his pulse rate in the first three days on a general diet, and in the first five days of the "trial diet."

It is seen in Chart 4 that his maximal pulse rate in the three days previous to the beginning of treatment was not very high—86. However, on an exclusive diet of beef on the fifth day, and of beef and milk on the sixth day (Chart 5), the pulse rate did not pass above 74. Following the addition of wheat on the seventh day the maximal rate reached 90, and he had a headache. He took no wheat after the evening meal of the seventh day, and there

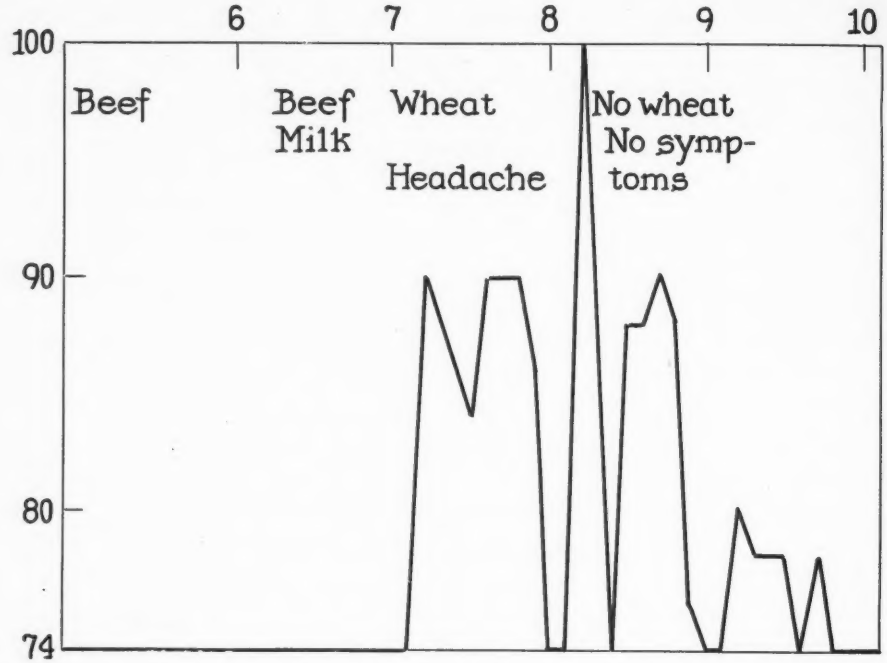

Chart 5.—The course of the pulse rate in patient J. G. on the first five days of the trial diet.

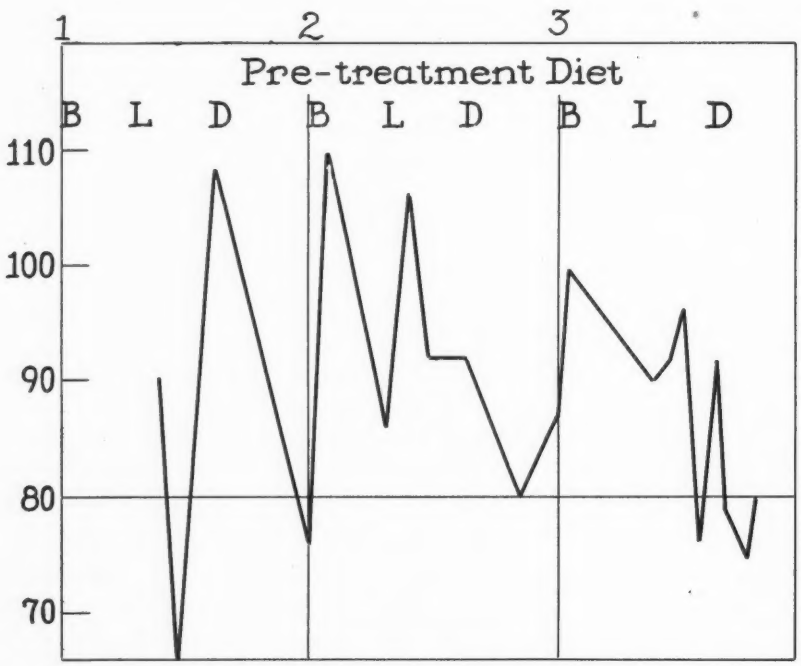

Chart 6.—The course of the pulse rate in patient C. T. on three days prior to the period of trial diet. *B*, breakfast; *L*, lunch; *D*, dinner.

were no symptoms thereafter. There was a vigorous recurrent tachycardia on the eighth day and a milder one on the ninth. The subsequent trial of coffee caused hives, but no headache. Cane sugar caused headache without hives. This patient has made trials of at least twenty-four other foods, with no unexplained clinical symptoms nor tachycardia in the succeeding twelve months.

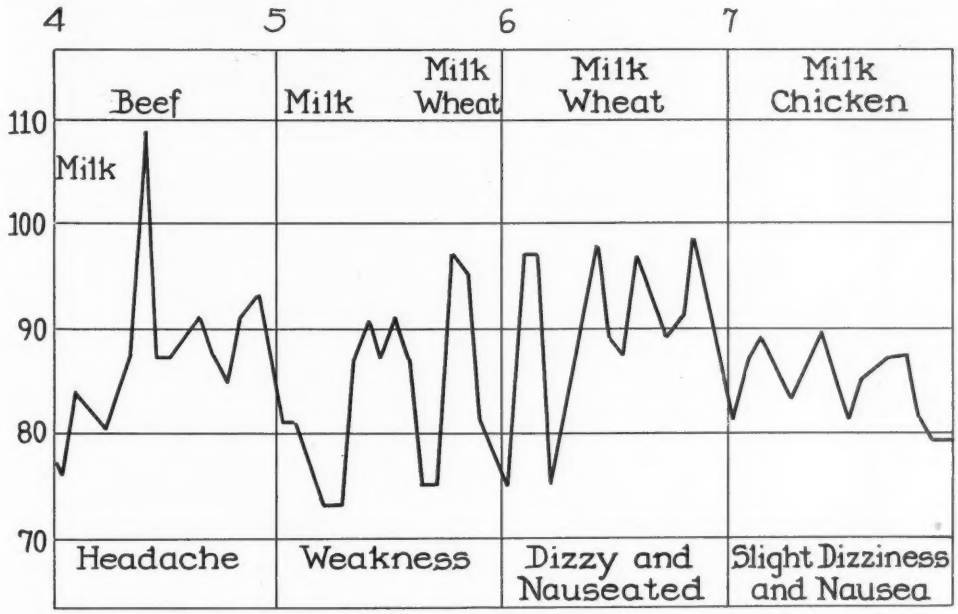

Chart 7.—The course of the pulse rate in patient C. T. on the first four days of the trial diet.

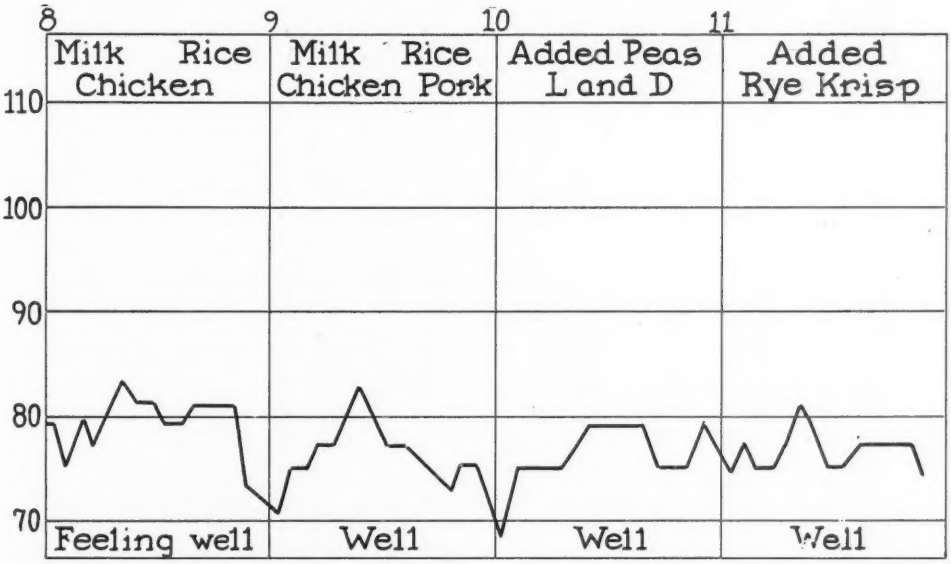

Chart 8.—The course of the pulse rate in patient C. T. on the second four-day period of the trial diet.

The cutaneous tests with the common inhalants and foods, including wheat and coffee (scratch tests with strong glycerinated extracts), were negative.

The record of the pulse rate in a young woman, C. T., employed by a large life insurance company in New York City, is shown in Charts 6, 7, and 8.

TABLE I

PATIENT	AGE	SYMPTOMS	RANGE OF PULSE RATE		TREATMENT BEGAN	ALLERGENIC FOODS
			BEFORE TREATMENT	AFTER TREATMENT		
E. F. C.	49	Migraine, mucous colitis, dizziness, neuralgia, nervousness, abdominal pain, physical tiredness, canker sores, intestinal bleeding, nausea, vomiting, "extrasystoles," angina pectoris	Up to 180	68 to 84	Aug. 1935	Beef, lamb, fish, egg, milk, cereals, string beans, Lima beans, white potato, sweet potato, tomato, onion, artichoke, squash, cabbage family, lettuce, carrot, beet, spinach, turnip family, nuts, plum, pear, melon, pineapple, peach, banana, apricot, asparagus, parsnip, pepper, coffee, pork, orange
A. F. C.	65	Migraine, gastric pain (cramplike), abdominal pain, pain in region of gall bladder, heartburn, canker sores, severe dizziness, physical tiredness, gastrointestinal bleeding, extrasystoles, psychic depression, nervousness, chronic rhinitis, constipation, neuralgia, conjunctivitis	36 to 100	58 to 72	Aug. 1935	Pork, fish, cereals, cane sugar, white potato, sweet potato, beet, carrot, spinach, pea, beans (string and Lima), cabbage family, lettuce, asparagus, parsnip, artichoke, turnip family, tomato, mushroom, maple sugar, chocolate, apple, olive, date, nuts, yeast, squash, pineapple, plum, peach, apricot, melon, orange, grapefruit, lemon, onions, grapes, pepper, coffee, tea, aluminum, fowl, cow's milk, banana
M. M. D.	27	Migraine, nausea, chest pain, marked physical tiredness, nervousness, vomiting, canker sores, constipation, bronchial asthma	70 to 100	70 to 80	Jan. 1936	Pork, potato, orange, coffee
A. R.	28	Migraine, nervousness, chest pain, heartburn, dizziness, extrasystoles, neuralgia	66 to 108	70 to 84	Aug. 1939	Milk, yeast
C. T.	26	Nausea, diarrhea, neuralgia, extrasystoles ("fluttery heart"), marked physical tiredness, canker sores, marked dizziness, psychic depression, constipation, "nervous and emotional instability—incurable"	65 to 112	72 to 78	Jan. 1940	Beef, cereals, cane sugar, orange, tomato, prune, grapefruit, potato, pineapple, onion, banana, strawberry, egg, lemon
J. G.	36	Incapacitating 3-day headaches at 1- to 2-week intervals; frequent urticaria and angioneurotic edema, heartburn	66 to 86	66 to 80	Jan. 1940	Wheat, sugar cane, coffee
H. E.	49	Dizziness, migraine, "gas," physical tiredness, anorexia	76 to 106	58 to 76	March 1940	Wheat, milk, coffee

W. W. F.	57	Headaches, heartburn, "indigestion," canker sores, marked physical tiredness, petit mal (frequent during past 20 years); epileptic sib	66 to 100	56 to 68	April 1940	Beef, orange, lamb
M. W. F.	52	Headaches, heartburn, "gas," indigestion, nervousness, marked physical tiredness	72 to 100	58 to 72	April 1940	Crisco, peanut, cinnamon, pecan
A. W. F.	17	Allergic sore throat	70 to 90	60 to 72	April 1940	Chocolate
F. C. F.	16	Severe headaches, dizziness, physical tiredness	58 to 84	58 to 70	April 1940	Chocolate, crisco
M. B.	22	Frequent spontaneous epistaxis	64 to 100	64 to 76	April 1940	Egg
P. W.	47	Constant severe headache, dizziness, marked physical tiredness, chronic rhinitis, postnasal drip, bronchial asthma ("infectious")	68 to 100	62 to 78	April 1940	Beef, lamb, egg, cow's milk
R. M.	22	Frequent severe headaches, dizziness, nervousness, canker sores, "indigestion," constipation, marked dysmenorrhea	68 to 100	62 to 78	May 1940	Beef, egg, potato, tomato, chocolate, coffee
A. P.	70	Marked generalized urticaria (dollar size), chronic cough, constipation	72 to 100	70 to 74	May 1940	Beef, cow's milk, egg, yeast, olive, corn, banana, proprietary laxative
S. I. H.	40	Frequent headaches	68 to 108	68 to 80	June 1940	Beef, lamb, white potato, tomato, orange, yeast, sugar cane
H. A. S.	58	Marked urticaria at long intervals, occasional headaches, canker sores, neuralgia, physical tiredness	60 to 105	60 to 74	June 1940	Beef, bean, asparagus, chocolate, corn, Swiss chard, peas, cinnamon, eggplant, coffee
J. F.	53	Headaches, angioneurotic edema, physical tiredness, chronic rhinitis, postnasal drip of 10 years' duration, constipation	68 to 108	68 to 80	June 1940	Lamb, egg, chicken, potato, tomato, grapefruit, chocolate, coffee, tea, orange
W. S. C.	41	Headaches, "indigestion," gas, nervousness, marked physical tiredness	46 to 70	46 to 62	July 1940	Beef, potato, cow's milk, tobacco
J. V.	35	Headaches (almost continual, incapacitating), asthma (mild), physical tiredness, chronic rhinitis	44 to 78	48 to 64	July 1940	Cow's milk, wheat, carrot, tomato, lettuce, banana, melon, lemon, peanuts, egg, peach, cereals, cabbage family, bacon, ham, pear, peppermint

TABLE I—CONT'D

PATIENT	AGE	SYMPTOMS	RANGE OF PULSE RATE		TREATMENT BEGAN	ALLERGENIC FOODS
			BEFORE TREATMENT	AFTER TREATMENT		
E. B.	50	Constant headache, nervousness, neuralgia; "very irritable," marked physical tiredness	82 to 108	72 to 76	July 1940	Wheat, pork
Dr. R.	26	Severe incapacitating headaches, physical tiredness, chronic rhinitis, dizziness, neuralgia, heartburn	70 to 90	70 to 76	Aug. 1940	Milk (cow's), egg, orange, grapefruit
R. F.	34	Severe headache (left side), heartburn, neuralgia, physical tiredness, nervousness, chronic rhinitis	64 to 114	62 to 76	Sept. 1940	Fowl, milk (cow's), wheat, corn, nuts, plum (prune)
A. F.	10	Headaches, bronchial asthma	82 to 124	74 to 78	Oct. 1940	Milk, beef, pork, squash, grape, peas, lettuce, tomato, cabbage family, celery, orange, peanut, marshmallow
NV'W	38	Severe headaches, indigestion, heartburn, canker sores, dizziness, physical tiredness, neuralgia, chronic rhinitis, nervousness	64 to 86	62 to 76	Nov. 1940	Pork, potato, sweet potato, tomato, pear, beef, cabbage family, peas, string beans, mushrooms
Dr. I. P.	46	Severe gastric pain (suspected ulcer) operation contemplated	84 to 104	72 to 78	Jan. 1941	Diagnosis incomplete Cow's milk, major allergen (if there are others they are negligible)
L. H. B.	20	Marked headaches, dizziness, physical tiredness, occasional urticaria, acne, nausea, vomiting	60 to 90	58 to 70	Jan. 1941	Wheat, chocolate, peas, onion, string bean, squash, white and sweet potatoes, spinach, rice, chicken, peach, apricot, orange, banana, tomato, grapefruit, pineapple, cabbage, carrots, lettuce, beets
J. J. V.	38	Headaches (frequent), bronchial asthma, nervousness, physical tiredness, acne, angioneurotic edema	70 to 94	62 to 74	Jan. 1941	Cow's milk, tomato, onion, potato, lamb, Lima bean, fish, oyster, tobacco, sweet potato, coffee, chocolate, orange, chicken, apple, banana, cabbage, celery
C. B.	23	Chronic rhinitis, bronchial asthma	72 to 100	67 to 72	Jan. 1941	Wheat, egg, lettuce, cane sugar
K. S.	48	Migraine, physical tiredness, constipation, nervousness, neuralgia, extrasystoles	70 to 110	70 to 80	Jan. 1941	Fish, lamb, potato, peas, string beans, carrot (tomato not tested but avoided on account of earlier experiences)
M. D. B.	Over 50	Heartburn, migratory neuralgia, constipation, physical tiredness	62 to 90	52 to 60	April 1941	Milk, orange, lemon, carrot, beet, asparagus (spinach not tested but avoided)

The outstanding symptoms in her case were recurring attacks of marked dizziness (without headache), which so interfered with her work as to make her continued employment uncertain. The company physicians had once placed her under psychiatric observation in a sanatorium for three months and had reached a diagnosis of "emotional and nervous instability" which they considered incurable. She consulted me only to inquire why she was always decidedly worse immediately after injections of "cold vaccine." At that time, under the threat of losing her position, and because her life was otherwise intolerable, she was contemplating suicide.

Since it was imperative that she be kept from her work not longer than ten days, more rapid additions to the diet were allowed than is usually advisable. The headache caused by beef (taken three times) was the first severe headache in her experience.

The first rise of the pulse rate on the fifth day was interpreted as recurrent from the reaction to beef; the second rise on that day was thought probably to be due to wheat. The patient has been found sensitive to all other cereals and sugar cane, and to orange, grapefruit, tomato, coffee, onion, banana, pineapple, strawberry, lemon, and egg.

During the succeeding nineteen months (to date) she has, with few exceptions,* been free from the dizziness as well as all the other minor symptoms (canker sores, nausea, diarrhea, "fluttering heart," neuralgia, and marked lassitude). The exceptional episodes could all be referred to the unwitting eating of one or the other of the allergenic foods. On one occasion she ate at the evening meal only the meat of a fowl that had been stuffed with bread, and the next day she suffered a mild dizziness.

Direct intracutaneous tests with extracts of the incriminated foods gave negative results. Intracutaneously injected into a known receptive patient (E. D.) her serum in a quantity of 0.05 c.c. failed entirely to sensitive the skin passively to beef, wheat, orange, and coffee (no tests were done with the other allergenic foods).

Table I presents the essential data of the 31 persons with nonreaginic food allergy who have been successfully treated with the method of trial diet controlled through the pulse rate.† The following points may be emphasized.

The range of the food allergic pulse rate varies greatly in different persons, for example, J. G. and F. C. F. showed high rates of only 86 and 84, respectively. The range of the normal pulse rate varies greatly in different persons; the high count (68) of W. W. F. is the same as the low count of J. F.

In most cases the symptoms mentioned in Table I could be induced by eating one or the other of the allergenic foods identified by means of the tachycardia. Many of those symptoms have not been generally recognized as due to food sensitivity; especially, nervousness, dizziness, allergic sinusitis, physical tiredness ("no 'count feeling"—Vaughan[3]) and irritability. *All of these symptoms disappeared in the respective patients after the pulse rate became normal following the elimination of the allergenic foods, and they were usually reproducible in all such persons by the mere eating of the forbidden foods.*

In one patient with food allergy (Dr. "C.") the tachycardia, although amply present (range of 64 to 84, once 90), could not be practically used in the detection of the offending foods. On the one hand, the single pulse rate of 90 was recorded on a day when the patient was "feeling quite well"; whereas, on one morning when he "awoke feeling as though an attack were impending," the pulse rate ranged between 72 and 76. However, this patient showed other peculiarities. Dr. "C.," with much difficulty using the Andresen method, has identified "meat, fish, chicken, eggs, wheat, chocolate, and coffee" as allergenic

*These will be discussed in a later publication.

†In each instance the pulse rates were noted by the patient, the figures representing the half-minute count multiplied by 2. No instructions were given the patients as to posture, etc., when the counts were being taken. This and other practical considerations will be discussed in detail in a later publication.

in his case. Yet, on one occasion after having eaten chicken liberally on three successive days (just after a four-day attack), he was "feeling quite well" on the third day—the day of the single pulse rate of ninety. By avoiding the above-mentioned foods, or eating them in the respective "latent period"—"occasionally, or once weekly"—he has reduced his attacks to "one severe one and one mild one in 5 months."

There are two possible explanations of this failure to arrive at complete relief of the allergic symptoms. (1) The patient, being a doctor of medicine was in the habit of taking certain commonly used drugs at the first premonition of an "attack." It has been seen in other cases that drugs may so affect the pulse rate as to interfere with the interpretation of the record. (2) This case was being studied long before the allergenic action of metals and essential oils had been recognized.*

There have been a few other "failures," all of which are similarly explainable.

Most of the foods that have been identified as excitants of nonreaginic food allergy in the 31 patients that have been under this kind of treatment have been common ones. Table II shows the list of these foods.

TABLE II

FOODS MOST COMMONLY INCRIMINATED AS EXCITANTS OF FAMILIAL NONREAGINIC FOOD ALLERGY IN 31 CASES

Wheat	11	White potato	8
Orange	11	Chocolate	8
Egg	11	Coffee	7
Beef	10	Sugar cane	6
Milk	10	Sweet potato	5
Tomato	9		

LATENT PERIOD IN NONREAGINIC FOOD ALLERGY

In the case of A. F. C. it had been noticed that after sensitivity to a number of vegetable foods had been unquestionably established in several separate tests, in all of which the allergenic food had been eaten in generous quantity several times over a period of one or more days, if the food was avoided for a long time it could always be eaten once, often more than once, with impunity. I began to generalize my thoughts about this recurring phenomenon after I came across the following statement by Vaughan[3a]: "This, we shall see, is very characteristic of food allergy, that an allergenic food may be eaten at times with impunity, at other times with consequent symptoms."

The thought took a practical turn upon the following observation of J. G. (see Chart 5), who had been found sensitive to wheat. About two months after his first test with wheat, he ate, only at one meal, an unstinted quantity of bread after which he suffered no clinical symptoms and observed no rise of the pulse rate above his normal maximum.

The relatively short interval (two months) between the two tests with wheat suggested the question how short the interval can be made without the occurrence of a reaction. This question was studied in the case of A. F. C., by administering at six- or seven-day intervals a number of vegetables and fruits to all of which he had been proved sensitive. Some of these foods (peas, string beans, Lima beans, beet, date, raisin) had not been eaten for several weeks, some not for several years (asparagus, broccoli, alligator pear, celery, apple, yam, squash, endive, tomato, onion, peach, pear, prune, blueberry, raspberry, and cane sugar). With three exceptions no tachycardia or allergic symptom followed the first two ingestions of these foods.

Broccoli caused no symptoms on the first ingestion, but after the second, six days later, there was heartburn and an eight-point rise of the pulse rate from the normal maximum of

*To be discussed in a later communication.

72. Squash on the first eating caused no reaction, but one week later it caused nervousness and a tachycardia (93). Yam, eaten for the first time after an abstinence of many years, caused headache and heartburn, the latter being felt within a few hours, and the former on the following morning.

Patient A. R. (see Charts 2 and 3), after abstaining from milk for six months, has taken a glass of milk once without experiencing symptoms or tachycardia.

The latent period is illustrated in the case of M. M. D. (Chart 1), who ate oranges from dinner January 16 to lunch January 18 before headache and tachycardia (to 104) began.

There was a similar experience with oranges in the case of C. T., symptoms being delayed for nearly forty-eight hours.

In some instances in the case of A. F. C. opportunity has been had to observe that the longer the interval of abstinence from an allergenic food the longer the latent period. Thus, string beans, after having been previously avoided for a number of years, were eaten almost every day from October 3, 1939, until March 10, 1940, when symptoms and tachycardia appeared, which ceased about twenty-four hours after the last consumption of string beans. On April 13 eating of string beans was resumed; at first at four- to five-day intervals and from May 22 at seven-day intervals without symptoms until June 19, when mild headache appeared in the night, and again June 26, when abdominal pain followed soon after eating the beans. After an interval of two weeks, indulgence in a liberal quantity of the beans was followed shortly by slight intestinal discomfort. The pulse rate was 72 after walking up two flights of stairs.

Grapes and raisins had been previously avoided for about nine years, when after January 10, 1940, they were taken almost daily without symptoms, until February 12, on which date heartburn, flatus, and headache appeared. Lettuce, which was also being eaten, was discontinued on February 16; but the headaches, heartburn, and flatus continued until twenty-four hours after grapes and raisins were omitted on February 28. There were no allergic symptoms after February 29 until March 4 (heartburn from rice wafers) and March 10 when headache marked the return of sensitivity to string bean.

From the foregoing data it is seen that the latent period of nonreaginic food allergy is not constant. It varies in different persons and in the same individual with respect to different foods. The latent period has been encountered in 13 of the 31 patients (E. F. C., M. M. D., C. T., J. G., W. W. F., R. M., H. A. S., J. F., W. S. C., A. R., R. F., M. D. B., L. H. B.). Most of the 13 patients are making regular practical use of the phenomenon by eating the respective allergenic foods at safe intervals.

The phenomenon of the latent period is clearly described by Vaughan,[3a] who cites in illustration a case of sensitivity to egg with avoidance of that food "for three years." The patient, being found "negative" to the cutaneous test, resumed the eating of egg and, one month later, began again to exhibit the allergic symptom (migraine). In such a case Vaughan speaks of the allergenic food as a "build-up" food, referring to the time required, after the period of avoidance, for the re-establishment ("build up") of the sensitivity.

SIGNIFICANCE OF THE LATENT PERIOD OF NONREAGINIC FOOD ALLERGY

An interpretation of the latent period of food allergy is found in a simple description of the fact in other words; namely, the sensitivity is lost for a time, the duration of which depends probably among other things, upon the length of the period of avoidance.

One may say that in many instances continual contact with the excitants of nonreaginic food allergy is necessary for the maintenance of that kind of human allergic disease. Vaughan evidently entertains the same view of the phenomenon.

This phenomenon is seldom, if ever, observed in reaginic atopy. It is true that reaginic atopy is sometimes "outgrown" but not necessarily following avoidance. Indeed, it seems well established that the natural process of outgrowing hay fever is often expedited by artificially increasing the exposure to the excitants of it. Under the specific therapy of hay fever by injections of an extract of pollen, the reagin content of the blood diminishes in many patients after a few years of continual (perennial) treatment.[4]

Thus the "latent period" of nonreaginic food allergy stands as a mark of differentiation of this category from the atopic group.

SUMMARY

1. Evidence has been presented of an association of tachycardia with clinical symptoms of familial nonreaginic food allergy, which was so regular in the 31 cases studied that the tachycardia could be used as an objective diagnostic criterion of the allergic reaction through which the specific excitants (foods) could be identified.

2. A "latent period" of lost sensitivity has been recognized in a number of instances of food allergy. In some instances this latent period has been found to vary in duration according to the length of the period of avoidance of the respective allergenic food. The practical use of the latent period is described.

3. Some of the grounds for distinguishing two categories of familial sensitivity are discussed.

4. The occurrence of reaginic and nonreaginic sensitivity to foods in the same individual has been described, and the practical consequences of such a coincidence are discussed.

Addendum (August 25, 1941).—The editors of this JOURNAL have permitted me to withhold this note until the article was about to be printed, in order to extend the period of observation of the patients.

The clinical results can be summarized as follows:

(1) All patients, except M. B., are still under occasional observation.

(2) All patients have had voluntary and blind tests of their specific sensitivity to various (for them) allergenic foods since the completion of their course of treatment by trial diet. In some instances no tachycardia nor allergic symptom followed the test (latent period). In all the other instances there was tachycardia, usually with one or more of the listed allergic symptoms. In most cases the test did not cause *all* the allergic symptoms. Thus, C. T. sometimes suffers tiredness or leg ache without headache or dizziness; sometimes headache alone, sometimes dizziness alone, sometimes both together. These different combinations of symptoms were present on different days under test with a single allergenic food (cane sugar). All symptoms disappeared after that food was eliminated from the diet.

(3) No unexplained allergic symptoms have occurred in any of the patients that have remained under observation.

(4) Some of the patients frequently retest themselves or deliberately "indulge" themselves if the resulting symptoms are not incapacitating; they are able to control their earlier neurasthenia, knowing the cause of each allergic episode.

(5) In some instances one or more of the symptoms that had been exhibited previous to the dietary treatment did not recur at any of the subsequent tests. For example, the monthly petit mal seizures of patient W. C. F. have not recurred once since April, 1940, although beef has been eaten occasionally, causing a moderate tachycardia.

The foregoing summary is, in a sense, an understatement; scientific language does not lend itself to the faithful portrayal of the great improvement described by many of these patients in the state of their "general health," in their sense of "well-being," in their

outlook on life, and in their behavior toward their associates. Of the great importance of these imponderables I am convinced.

Further clinical details of these cases and additional cases, as well as a detailed description of the method of treatment, will be published in a forthcoming monographic report (C. C Thomas, Springfield, Ill.).

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LEFT VENTRICULAR FAILURE DUE TO ESSENTIAL HYPERTENSION*

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THE most common forms of circulatory failure encountered in the office of the general practitioner are those in which the onset is with failure of the left side of the heart.[1] Failure of the left ventricle results from diseases in which the work of the chamber is increased or its muscle is diseased, and includes such causes as essential and nephritic hypertension, arteriosclerosis, and syphilitic disease involving the orifices of the coronary arteries to the left ventricle, defects of the aortic valve, and mitral disease with predominant regurgitation. Essential hypertension is the most common etiologic factor in the causes of organic heart disease,[2] and probably accounts for the greatest number of cases of isolated left ventricular failure.

Except as described by Hope,[3] the concept of isolated failure of the left ventricle of the heart is one that has arisen within recent years.[4-6] White[6] stated that failure of the left ventricle is more common and important than that of the right ventricle and should be looked for zealously, since by early recognition and proper treatment not only may heart failure of the left side be relieved, but heart failure of the right side, which is likely to follow, may be prevented or postponed.

In a previous study on the types of hypertensive heart failure[7] in 594 patients it was found that isolated failure of the left ventricle occurred in 156 (26.5 per cent) of the cases. These patients had no signs of systemic congestion. The mortality was lower than among those with combined ventricular failure, and the leading cause of death was not the usual congestive heart failure.

The present clinical study comprised 190 patients with isolated failure of the left ventricle due to uncomplicated essential hypertension. It included 150

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males (79 per cent) and 40 females (21 per cent), whose ages varied from 35 to 77 years at the time of onset of the cardiac symptoms (Table I). Of this group 70.5 per cent were in the 40 to 60 year age group, the so-called "hypertensive age." In a previous study of all types of hypertensive heart disease, 78.8 per cent were in this age group.[8]

TABLE I

PERCENTAGE OF THE AGE GROUPS AT THE ONSET

AGES	MALE	FEMALE	TOTAL	PER CENT
31-40	2	7	9	4.7
41-50	42	13	55	28.9
51-60	67	13	79	41.6
61-70	36	6	42	22.2
71-80	4	1	5	2.6
Totals	150	40	190	100.0

Symptoms which indicated the onset of failure of the left ventricle were dyspnea, cardiac pain (precordial or epigastric or both), weakness, and palpitation. Cardiac pain was a solitary complaint of 20 (10.5 per cent) of the 190 patients, but in all others the cardiac pain, weakness, and palpitation were associated with dyspnea (Table II). White[6] indicated several important symptoms and signs that point to weakness and failure of the left ventricle, which, he contended, should permit its recognition in the absence of mitral stenosis and congenital heart disease: They are (1) cardiac dyspnea, (2) cardiac asthma or acute pulmonary edema, (3) diminishing vital capacity due to heart disease, (4) engorgement of the roentgen shadows of the lung hilus blood vessels, (5) protodiastolic gallop rhythm at the apex in the absence of heart block, (6) pulsus alternans, and (7) increasing accentuation of the pulmonary second heart sound. Of these seven points the symptoms of dyspnea and cardiac asthma, and the signs of acute pulmonary edema, were the most reliable and helpful in the diagnosis of failure of the left ventricle due to essential hypertension.

TABLE II

FREQUENCY OF THE IMPORTANT SYMPTOMS AND THEIR DURATION

DURATION	DYSPNEA AND CARDIAC PAIN	DYSPNEA	CARDIAC PAIN	DYSPNEA AND WEAKNESS	DYSPNEA AND PALPITATION
1 day to 6 months	50	34	18	7	3
7 months to 1 year	14	6	0	3	3
2 to 5 years	22	14	2	2	3
6 to 10 years	3	6	0	0	0
Totals	89	60	20	12	9
Percentage	46.8%	31.6%	10.5%	6.3%	4.8%

Combined dyspnea and cardiac pain were the most common complaints (46.8 per cent) at the onset of failure of the left ventricle. The mortality was lower among these patients than among those who had the same complaints on the basis of combined failure of both ventricles. The lowest mortality occurred among those who complained solely of dyspnea (31.6 per cent) or of cardiac pain alone (10.5 per cent). Among these patients the mortality was only 13 per cent. Thus the importance of recognizing failure of the left ventricle early,

as either dyspnea or cardiac pain, alone or combined, are the most important symptoms, cannot be overemphasized from the standpoint of attempting to reduce the immediate mortality.

The duration of the failure of the left ventricle, from the onset of the first cardiac symptoms, was estimated in 44 (23.1 per cent) known deceased patients (Table III). Data on 146 (76.9 per cent) living patients were added for comparison. Of the deceased 83.8 per cent died within two years after the onset of cardiac symptoms. The percentage of patients still living, who were observed within two years after the onset of symptoms, was 80.7 per cent, approximately the same ratio.

TABLE III

DURATION OF DISEASE AFTER ONSET OF CARDIAC SYMPTOMS

DURATION	DEAD				LIVING			
	M.	F.	TOTAL	%	M.	F.	TOTAL	%
1 day to 6 months	25	3	28	63.6	61	17	78	53.5
7 months to 1 year	4	1	5	11.4	19	6	25	17.1
2 to 5 years	6	3	9	20.4	28	3	31	21.2
6 to 10 years	2	0	2	4.6	5	7	12	8.2
Totals	37	7	44	100.0	113	33	146	100.0
Percentage	23.1%				76.9%			

Table IV indicates the age at death of these patients; 72.9 per cent died between 40 and 60 years, the "hypertensive age." In the same age period 76.6 per cent of the entire group with hypertensive heart disease[8] died, which was about the same proportion.

TABLE IV

PERCENTAGE OF THE AGE GROUPS AT DEATH

AGES	MALE	FEMALE	TOTAL	PER CENT
31-40	–	1	1	2.2
41-50	9	1	10	22.9
51-60	19	3	22	50.0
61-70	8	1	9	20.4
71-80	1	1	2	4.5
Totals	37	7	44	100.0

CONGESTIVE HEART FAILURE

In general, congestive heart failure was the cause of death in 65 per cent of deceased patients with hypertensive heart disease.[8] Among those with isolated failure of the left ventricle the common cause of death was equally divided between coronary thrombosis and congestive failure (Table V). Among 159 cases of hypertensive heart disease with established auricular fibrillation, congestive failure caused 85 per cent of the deaths;[9] in 36 cases with bundle branch block, it was the cause of death in 97.2 per cent of the deceased;[10] and among 127 patients with hypertensive heart with gross arteriosclerosis, it was the cause of death in 72 per cent of the deceased.[11] Since death occurred in only 16 (8.4 per cent) of the 190 cases during the period of observation, congestive heart failure could not be considered a common termination in these patients with isolated failure of the left ventricle. However, it was the cause of death, within six months of the onset, in each of the 16 patients.

TABLE V

PERCENTAGE OF THE CAUSES OF DEATH IN 44 PATIENTS

CAUSE	MALE	FEMALE	TOTAL	PER CENT
1. Coronary occlusion	15	1	16	36.4
2. Congestive heart failure	11	5	16	36.4
3. Uremia	6	0	6	13.6
4. Cerebral hemorrhage	5	1	6	13.6
Totals	37	7	44	100.0

Five illustrative cases are presented to demonstrate that in the absence of any irregular rhythms, complications, or advanced coronary arterial disease, and in spite of treatment, a certain small number of patients with failure of the left ventricle due to essential hypertension died from congestive heart failure.

CASE REPORTS

Patient 1. F. P., a 54-year-old white female, was known to have had hypertension and diabetes for six years. Her blood pressure was systolic 200 and diastolic 100, and the transverse diameter of her heart was 14 cm. at the onset of the first attack of acute failure of the left ventricle fifteen days before her death. In spite of bed rest and adequate doses of digitalis and diuretics, she died of congestive heart failure. Autopsy revealed only a moderately hypertrophied heart, which weighed 490 Gm., hyperemia and edema of the lungs, and cloudy swelling of the myocardium.

Patient 2. H. D., a 54-year-old white male, was known to have had hypertension for five years. At the onset of acute failure of the left ventricle five days before his death the blood pressure was systolic 292 and diastolic 154. There were crepitant râles at both lung bases, and the transverse diameter of his heart was 19 cm. The liver was not palpable and no edema was noted. Following bed rest, and the administration of digitalis and diuretics, no improvement was noted. Five days after the onset he died suddenly. Autopsy revealed a marked hypertrophy of the heart, which weighed 720 Gm., and mild sclerosis of the coronary arteries with occasional fatty and calcified plaques. There was marked edema of the lungs and passive congestion of the liver and spleen. Death was apparently due to congestive heart failure.

Patient 3. A. F., a 56-year-old white male, complained of dyspnea and weakness of three weeks' duration. He had had hypertension for one year. His blood pressure was systolic 210 and diastolic 160. Crepitant râles were present at the bases of both lungs, and the transverse diameter of his heart was 20 cm. After ten days of bed rest, digitalis, and diuretics, he failed to improve. When seen again six weeks later, his blood pressure was systolic 240 and diastolic 160, congestive heart failure was present and persisted until his death within three months. He lived five and one-half months after the onset of isolated failure of the left ventricle. The cause of death was congestive heart failure.

Patient 4. P. D., a 55-year-old white male, known to have had hypertension for four years, complained of dyspnea and precordial pain of five months' span. His blood pressure was systolic 210 and diastolic 160, and the transverse diameter of his heart was 18 cm. The liver was not palpable and no edema was noted. After bed rest and treatment with digitalis he became ambulatory in two weeks. Two months later, in spite of continued digitalis medication, he developed congestive heart failure and died within ten days. Death occurred eight months after the onset of isolated failure of the left ventricle. Congestive heart failure was the cause of death.

Patient 5. R. M., a 58-year-old white male, complained of dyspnea and weakness for eight months. His blood pressure was systolic 250 and diastolic 130, and the transverse diameter of his heart was 19 cm. The liver was not palpable and no edema was noted. His condition did not improve after bed rest, and the administration of digitalis and diuretics. Death occurred eight months and three weeks after the onset of isolated failure of the left ventricle, and was due to congestive heart failure.

CORONARY THROMBOSIS

As a cause of death coronary thrombosis or occlusion was common among the 190 patients with failure of the left ventricle; very common, one might add, as compared with the other groups of patients with hypertension. In general, it occurred as the cause of death in 10 per cent of all deceased hypertensive patients.[8] Among those with established auricular fibrillation it was noted in only one case (2.8 per cent).[9] In the cases with bundle branch block it did not occur at all.[10] And among those who had hypertension with gross arteriosclerosis, where coronary occlusion was common, it was the cause of death in only 6.6 per cent of those who died.[11]

Coronary thrombosis appears to be the important factor in failure of the left ventricle due to essential hypertension. It was the leading additional factor (8.5 per cent) in 190 patients (Table VI). During the course of hypertensive heart disease[8] it occurred as an additional factor in 6 per cent of the patients; in those with auricular fibrilation[9] it was noted in only three (1.9 per cent) of the 158 patients; and only one patient (2.7 per cent) with bundle branch block[10] had coronary occlusion. However, in persons with hypertensive heart disease with gross arteriosclerosis, coronary occlusion was more common, since it occurred among 9.5 per cent of 127 patients.[11] This was to be expected since the patients in the latter group were older, 84 per cent were over 60 years, and sclerosis of the coronary arteries was the rule rather than the exception.

TABLE VI

ADDITIONAL FACTORS IN 190 PATIENTS

CONDITION	MALE	FEMALE	TOTAL	PER CENT
1. Coronary occlusion	13	3	16	8.5
2. Angina pectoris	10	2	12	6.3
3. Cerebral hemorrhage	8	2	10	5.3
4. Diabetes mellitus	4	1	5	2.6

Cases of isolated failure of the left ventricle with regular rhythm, where death occurred due to the coronary factor, are added to emphasize and illustrate the facts mentioned.

CASE REPORTS

Patient 1. M. W., a 54-year-old white male, had attacks of pain localized strictly to the precordial area for six months. These attacks increased in frequency, and gradually he became short of breath. When he was first examined eleven days before his death, his blood pressure was systolic 190 and diastolic 140. Crepitant râles were heard at the bases of both lungs. The transverse diameter of the heart was 17 cm. The liver was not palpable and no edema was noted. Following bed rest and treatment with digitalis, his condition improved, but on the eleventh day he suffered a typical attack of acute coronary thrombosis. His blood pressure dropped to systolic 146 and diastolic 92, and he died on the same day. An autopsy revealed severe coronary sclerosis, with marked narrowing of the coronary lumen. There was a recent thrombus occluding the left descending branch of the coronary artery at the site of an atheromatous ulcer and a recent myomalacia in the anterior wall of the left ventricle. The heart weighed 720 Gm. and was markedly hypertrophied. This was a definite case of isolated failure of the left ventricle on the basis of essential hypertension. Death was due to coronary thrombosis while the patient was under treatment and showed marked improvement.

Patient 2. S. G., a 50-year-old white male, complained of increasing dyspnea and precordial pain of one month's duration. His blood pressure was systolic 220 and diastolic 180, and he had crepitant râles at the bases of both lungs. The liver was not palpable and no edema was noted. The transverse diameter of the heart was 15 cm. He was given adequate doses of digitalis, and his symptoms disappeared on ambulatory management. One month from the day he was first seen, and two months after the onset of symptoms of isolated faliure of the left ventricle, he suffered a severe attack of precordial pain, his blood pressure dropping to systolic 130 and diastolic 100; a loud pericardial friction rub gradually became audible over the entire precordium. Death occurred six days after the onset of coronary thrombosis. Autopsy revealed a thrombosis of the anterior descending branch of the coronary artery with recent myomalacia. The heart, which was moderately hypertrophied, weighed 500 Gm.

Patient 3. R. O., a 62-year-old white male, was treated with digitalis for three years following complaints of dyspnea and substernal distress. At the time of the first examination his blood pressure was systolic 196 and diastolic 144, and the transverse diameter of his heart was 15 cm. Crepitant râles were noted at the bases of both lungs. The liver was not palpable and no edema was noted. He was ambulatory and continued at work during most of the period. Three years and one week after the appearance of isolated failure of the left ventricle he had an attack of coronary thrombosis. Death occurred three weeks after the coronary insult.

Patient 4. C. A., a 67-year-old white male, known to have had hypertension, had been on digitalis since the occurrence of failure of the left ventricle five years before at the age of 62. During the entire period he was ambulatory. The only findings were arterial hypertension, crepitant râles at the bases of both lungs (especially when he omitted the digitalis for a period of several weeks), and a transverse diameter of 15 cm of the heart. One day he developed a sudden severe pain in the epigastric region; this pain radiated to both shoulders and persisted. His blood pressure dropped to systolic 138 and diastolic 82, his heart tones became distant and muffled, and his temperature ranged from 99° to 101° F. Four days after the coronary assault he developed a left hemiplegia and lingered five weeks. Death occurred five years and five weeks after the first attack of isolated failure of the left ventricle.

Patient 5. I. S., a 51-year-old white male, had repeated attacks of failure of the left ventricle since the age of 46. He took digitalis as prescribed only during the periods of distress. His blood pressure was systolic 170 and diastolic 130, and he had crepitant râles at both lung bases. The transverse diameter of his heart was 15 cm. At no time did he have a palpable liver or edema of the lower extremities. Five years and four months after the first attack of failure of the left ventricle he had an acute coronary thrombosis. He died the same day.

AURICULAR FIBRILLATION

Auricular fibrillation occurred in 38 (20 per cent) of 190 patients with isolated failure of the left ventricle, as compared with 25.3 per cent of the 623 patients with hypertensive heart disease previously studied.[9] The age and sex ratio of the two groups were the same. Of the patients in this series, only six (15.8 per cent) of the 38 with auricular fibrillation died as compared with 38 (25 per cent) among the 152 with regular rhythm. Five deaths among those with the irregular irregularity were due to congestive heart failure, while the sixth was the result of a cerebral hemorrhage.

CASE REPORTS

Patient 1. M. S., a 38-year-old-white female, complained of dyspnea and precordial pain for four months. Her blood pressure was systolic 146 and diastolic 116, and the transverse diameter of her heart was 16 cm. Auricular fibrillation was present. In spite of bed rest and treatment with digitalis she died within ten days, apparently due to congestive heart

failure. Autopsy revealed moderate hypertrophy of the heart, which weighed 510 Gm., multiple recent hemorrhagic infarcts in the right lung, and passive congestion of the liver, lungs, and spleen.

Patient 2. F. H., a 69-year-old white female, complained of dyspnea of one year's duration. Her blood pressure was systolic 198 and diastolic 104, and the transverse diameter of her heart was 20 cm. Moist râles were heard at the bases of both lungs. Auricular fibrillation was present. Death occurred on the second day of treatment, apparently due to congestive heart failure. Autopsy revealed a moderate hypertrophy of the heart, which weighed 550 Gm., and congestion of the lungs, liver, and spleen.

Patient 3. G. W., a 57-year-old white male, complained of dyspnea and palpitation of eleven months' duration. His blood pressure was systolic 154 and diastolic 106, and the transverse diameter of his heart was 21 cm. Auricular fibrillation was present. Crepitant râles were audible at both lung bases. In spite of bed rest and administration of digitalis he died within three weeks, approximately one year after the onset of symptoms. The cause of death was congestive heart failure. Autopsy revealed a markedly enlarged heart, which weighed 760 Gm., and passive congestion of the kidneys, lungs, liver, and spleen.

Patient 4. J. M., a 60-year-old white male, had an attack of failure of the left ventricle at the age of 55, at which time the blood pressure was systolic 186 and diastolic 130. During this period he continued on treatment with digitalis intermittently. One month before his death he complained of increasing dyspnea and precordial pain. The transverse diameter of the heart was 16 cm. Moist râles were present throughout both lung bases. Auricular fibrillation was noted. The patient died one month after the second attack of severe failure of the left ventricle, his death being due to congestive heart failure.

COMMENT

Early recognition of isolated failure of the left ventricle due to essential hypertension requires careful evaluation[12] of the important symptoms, the most common of which are dyspnea and cardiac pain, singly or in combination. The cardiac manifestations, especially basal pulmonary congestion or unilateral hydrothorax, must be looked for, otherwise they will be overlooked.[13] The prognosis seems most favorable among patients with hypertension who complain solely of dyspnea or of cardiac pain alone because of the low mortality. It is important to recognize failure of the left ventricle early and to be able to diagnose the condition, since treatment by the administration of digitalis, by rest, or by both these measures is usually of great help, even though the heart rhythm is normal and there is no evidence of congestion of the systemic veins.[6] Many patients incapacitated from their occupation by exertional dyspnea are enabled to return to it by the administration of digitalis. Fishberg[14] mentions no other drug in this connection for the treatment of isolated failure of the left ventricle.

Digitalis (powdered leaf) was administered to all the 190 patients in doses ranging from 0.1 Gm. (1.5 grains) once daily to three times daily with good results. However, a certain few patients died of congestive heart failure despite the bed rest and digitalis, regardless of the rhythm being regular or irregular. As compared with patients with hypertensive heart disease who had failure of both ventricles, established auricular fibrillation, or bundle branch block, the patients with isolated failure of the left ventricle showed a much lower mortality (36.4 per cent) due to congestive heart failure.

Fahr[15] stated that coronary arteriosclerosis of some degree is found in 90 per cent of all hearts of patients dying with hypertension; this, he continued,

is a most important finding because it is a powerful contributing factor in the heart failure that follows high blood pressure, for hypertension and coronary arteriosclerosis are so intimately and frequently associated that they should be considered together. In discussing the treatment of failure of the left ventricle, Smith[16] expressed the opinion that an inadequate coronary circulation is commonly a prominent factor in the production of cardiac disability in hypertension.

Although coronary thrombosis or occlusion was more common (16.8 per cent) in patients with hypertension with failure of the left ventricle than in any other group of patients with hypertension, its frequency did not suggest that an inadequate coronary circulation was the prominent factor in the production of the cardiac disability.

A valuable clue to the analysis of this complicated problem is found in studies of the relationship between the myocardial fibers and capillaries. In hypertrophy of the heart due to hypertension and other forms of heart disease, the myocardial fibers enlarge proportionally, without any compensating multiplication of capillaries. Thus the actual value of cardiac tissue supplied by each capillary is increased in proportion to the degree of cardiac enlargement. This theory has been established on a firm basis by the experimental studies of Roberts[17-20] on human hearts, and of the Shipleys,[21] working in Wearn's laboratory, on rabbit hearts. Many years ago the hypothesis was suggested by Christian and by others before him in varying forms. Recently, Gross and Spark[22] have correlated the heart weight with counts of the number of arterioles in a unit area, without the careful control of experimental conditions used by the other workers. These studies suggest the possibility that the failure of the left ventricle, with the disturbed relation between muscle fiber size and the volume of blood flow through the capillary bed, may be the prominent factor in the production of the coronary thromboses. Any factor interfering with the nutrition of the muscle fiber will impair the contractility of the muscle fiber. Such loss of contractility may produce acute and chronic congestive heart failure.

Digitalis, as the factor which might have precipitated the coronary thromboses, could be readily excluded, since its effect is to aid the disturbed relationship and return it to balance rather than to increase the disturbance. The diuretics (theobromine sodiosalicylate, theocalcin, theophylline, and theophylline with ethylenediamine), which are supposed to increase the coronary circulation, cannot be indicted as the precipitating factors in the coronary thromboses. Apparently, the metabolic disturbance in the cardiac muscle when the left ventricle fails due to essential hypertension is such that some patients die from congestive heart failure, and others, with equal frequency, die of coronary occlusion. This might be the explanation for the more frequent occurrence of coronary thrombosis among persons with hypertension who have moderate to marked sclerosis of the coronary arteries, and the comparative absence of infarction in those who have no or only negligible coronary involvement. It is usually the latter group of patients with hypertension, who go on to congestive heart failure, a state in which, conversely, coronary thrombosis seldom occurs. The comparative absence of coronary occlusion among persons with hypertension with established auricular fibrillation supports this hypothesis, especially where the irregularity precedes and precipitates congestive heart failure.

The prognosis of those patients with failure of the left ventricle and auricular fibrillation was better than among those who had a regular rhythm, but only because of the absence of coronary thrombosis in the fibrillators. When coronary thrombosis occurred, as it did in 32 (16.8 per cent) of 190 patients, the prognosis was not good as 16 (50 per cent) of 32 died as the direct result of the thrombosis. The factor of coronary involvement leading to death must be kept constantly in mind when patients with isolated failure of the left ventricle due to essential hypertension are under treatment; especially is this true if the rhythm remains regular. However, there is the consoling fact that the lowest mortality among all classes of patients with hypertension is seen among those who have an onset with isolated failure of the left ventricle.

SUMMARY

1. Isolated failure of the left ventricle due to essential hypertension, the type that has a lower mortality than the combined failure of the right and left ventricles (congestive heart), is reported in 190 patients, the great majority of whom were males in the "hypertensive age."

2. Dyspnea and cardiac pain, alone or combined, were the leading symptoms at the onset in 89 per cent of the patients; in the other 11 per cent dyspnea was present along with palpitation or weakness.

3. Congestive heart failure, the common cause of death among patients with hypertensive heart disease, accounted for only 36.4 per cent of the deceased, but the duration of life after the onset of the congestive failure was less than six months in each of the 16 patients.

4. Coronary thrombosis, which was the cause of death in 10 per cent of patients with hypertension who died with all types of cardiac failure, and in only 6.6 per cent of those with gross arteriosclerosis, accounted for 36.4 per cent of the deceased in this series.

5. Auricular fibrillation occurred in 38 (20 per cent) of the 190 patients, and was the most common arrhythmia; the mortality was lower than in those with regular rhythm due to the absence of coronary thrombosis.

6. The studies of Roberts, Wearn, and the Shipleys suggest that the occurrence of failure of the left ventricle in essential hypertension, due to the disturbed relation between muscle fiber size and the volume of blood flow through the capillary bed, may be the precipitating factor of the coronary thrombosis, especially where coronary sclerosis already exists.

7. Coronary thrombosis, according to this analysis, should be kept in mind constantly during the treatment of isolated failure of the left ventricle in essential hypertension, and especially if the rhythm is and remains regular. Nevertheless, these patients with hypertension, on the whole, have a better prognosis than those who developed the combined type of hypertensive (congestive) heart failure where the occurrence of coronary thrombosis is a rarity.

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ACUTE EDEMATOUS PANCREATITIS*

A Case Presentation and Clinical Discussion of the Serum Amylase Test

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ACUTE edematous pancreatitis[1] is a disease often associated with chronic cholecystitis and cholelithiasis, and more often mistaken for it. However, a carefully regarded history and physical examination, supplemented by pertinent laboratory studies, will in most cases provide for a correct diagnosis. The recent literature has shown an ever-increasing school of thought which believes that the well-established picture of acute pancreatitis may truly be more variegated or subtle in character. In its milder, and perhaps more prevalent, role[2] of acute edematous pancreatitis, this clinical syndrome is one of benignity, and frequently subsides rather than progresses to the fulminant stage of hemorrhage and necrosis. However, the repeated attacks which have a tendency to occur may subsequently result in the well-known malignant phase of this lesion. Thus, interval cholecystectomy, a procedure designed to remove at least one of the more constant factors of pathogenesis, seems to be the operation of choice in the presence of biliary tract disease. It is very apparent from the literature that there is little present agreement as to the comparative value of the operative versus the conservative measures of treatment.

The present lack of diagnostic uniformity may be traced in part to the disregard of data obtainable by blood and urine amylase or lipase determinations. The amylase test in some quarters has unwisely fallen into disrepute, but when carefully performed in accordance with the fundamentals of enzyme chemistry,[3] it has been shown to be a practical, rapid, and reliable diagnostic aid when used within its limitations.

CASE REPORT

S. B., a 34-year-old white, married female, entered the Beth Israel Hospital complaining of severe upper abdominal distress of two and one-half days' duration. The history was obtained through an interpreter, since the patient had only been in the United States five weeks.

Present Illness.—Two and one-half days before admission the patient was seized by a sudden severe upper abdominal pain that occurred shortly after dinner. The pain seemed localized in both the epigastrium and the right upper quadrant and was present posteriorly as well. Nausea, repeated vomiting, and anorexia soon followed. The day before admission the intensity of the abdominal pain became sufficiently severe to have a physician called. He afforded the patient temporary relief with a hypodermic injection. On the day of admission the nature of the pain was unchanged—constant and not cramplike, the nausea and vomiting persisted, and upon a physician's advice the patient entered the hospital for diagnosis and treatment. Jaundice was not noted by the patient. There was no history of diarrhea, steatorrhea, or clay-colored stools. The pain in the back did not radiate to the shoulders or to either scapula.

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Past History.—The patient had always enjoyed rather good health. Within the past three years she had several recurrent attacks of upper abdominal pain similar to her present illness, but of milder character. Nausea, vomiting, anorexia, and jaundice were present. An intolerance for fatty foods was noted. There was neither history of alcoholism nor manifest foci of infection.

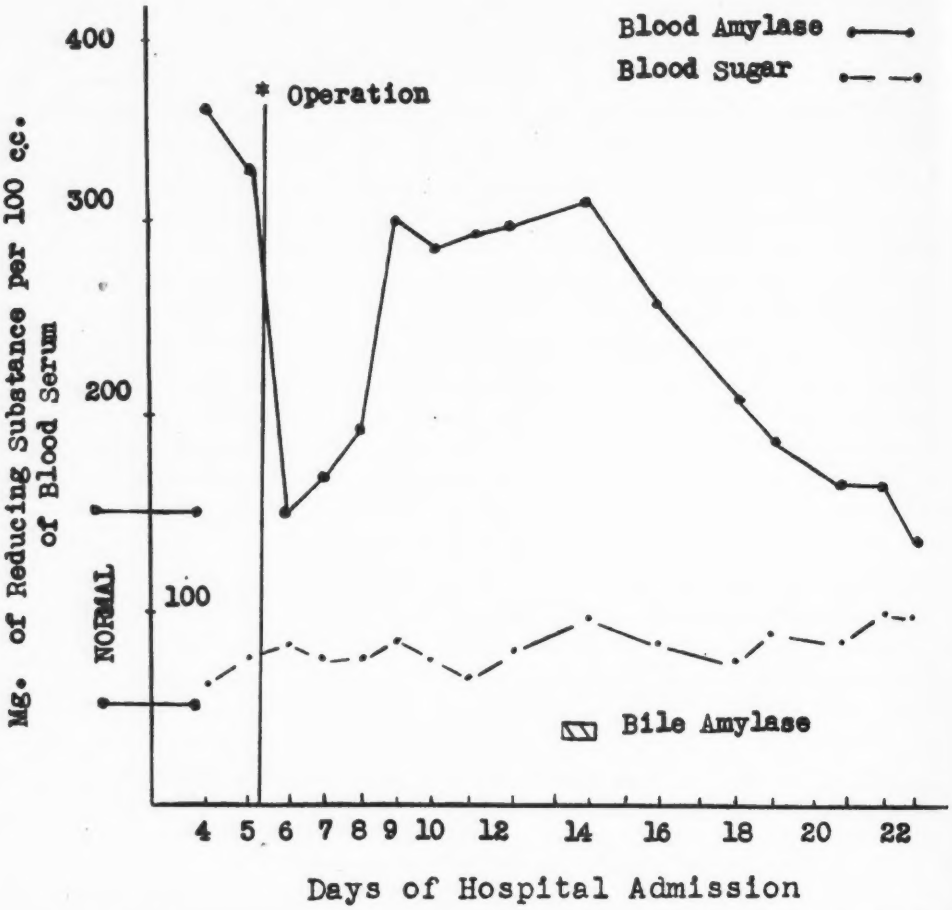

Fig. 1.—Sixteen serum amylase determinations with concomitant blood sugar levels. The immediate postoperative fall of the serum amylase value may either be the result of biliary decompression or merely coincidental. The secondary rise is probably accounted for by the use of a long-limbed T-tube draining the common duct and impinging upon the orifice of the duct of Wirsung or by edema occluding it.

Physical Examination.—On admission the patient's temperature was 99.8° F. Her respiratory rate was 24, and her pulse rate was 100 per minute. Her blood pressure was 128 systolic and 88 diastolic. Her body was well developed and well nourished. She showed no cyanosis, shock, or dyspnea. A faint jaundice was present. Examination of the abdomen revealed tenderness most marked in the epigastrium, although several days later the point of maximum tenderness shifted to the left. A minimum of muscle spasm was present. No masses were felt. Right costovertebral angle tenderness was present.

Laboratory Studies.—On the second day of admission she showed red blood cell count 4,800,000, white blood cell count 11,000, hemoglobin 80 per cent, and icterus index 24.4. Bleeding and coagulation times were normal. The urine contained no reducing substance. Sixteen serum amylase determinations were performed (Fig. 1), the first one recorded on the fourth day of admission. Cholecystography by the oral method faintly visualized the gall bladder.

Operative Findings.—Through an upper right rectus incision the peritoneal cavity was opened and a glossy edema of the hepatoduodenal ligament and the surrounding omentum was first noted. No free peritoneal fluid was found. The pancreas felt enlarged but not firm. The gall bladder was not acutely inflamed or distended. Numerous small stones were felt within the gall bladder. The hepatoduodenal ligament was bluntly divided, and the pancreas was seen to be markedly edematous. A single area of fat necrosis was present. Aspiration biopsy of the pancreas was attempted. Excision of an enlarged lymph node in the hepatoduodenal ligament was performed. The common duct was explored. It was found to be somewhat dilated, but no stones were located. Separate cultures of the gall bladder bile, common duct bile, and aspirated pancreatic fluid were taken. A cholecystectomy and choledochostomy with T-tube drainage were performed and the wound was closed.

Pathologic Report.—The wall of the gall bladder showed a small amount of infiltration by both polymorphonuclear cells and mononuclear cells. No thickening or ulceration of the mucosa was noted. The excised lymph node and surrounding fat tissue showed a rather marked edema and some cellular infiltration. Bacterial stains revealed no organisms. No section could be obtained from the aspiration biopsy of the pancreas.

Bacteriologic Report.—Cultures taken from the gall bladder wall, gall bladder bile, common duct bile, and aspiration fluid of the pancreas were all positive for *Staphylococcus albus hemolyticus.* Cultures taken from the T-tube drainage of bile grew *Cl. welchii, Bact. coli,* and *B. pyocyaneus.*

Postoperative Course.—Early epigastric distress and disappearance of jaundice were noted. The T-tube was removed on the fourteenth postoperative day. The patient was discharged well with no wound drainage on the twenty-first postoperative day.

DISCUSSION

The clinical aspects of this patient present characteristics which, in their entirety, are rather distinctive of acute edematous pancreatitis. The onset of pain is usually sudden, severe, and constant, frequently in the epigastrium or upper abdomen, and with surprising frequency radiates to the back. A previous history of similar episodes, with or without jaundice, is commonly obtained. Nausea and vomiting are seldom absent. Physical examination reveals that epigastric or upper abdominal tenderness is almost invariably present and is frequently severe. Associated muscle spasm is often less marked and may be minimal or absent. The patient appears very sick. Abdominal distention and jaundice are signs of lesser importance.

The temperature, pulse rate, and respiration may show only little elevation, and the blood pressure usually remains unchanged. The leucocyte count may be increased, and the urine rarely reveals a glycosuria.

Of salient significance, however, is the laboratory examination for pancreatic enzymes. Most recent observers now agree that the determination of the amylase content of either blood or urine, when reliably performed as to method and technique, is a simple, precise, and practical method of indicating this type of pancreatic lesion.[4] Older and more complicated procedures have proved cumbersome, inadequate, or otherwise undesirable to be rapidly carried out on an acutely ill patient.

Sixteen determinations of the content of amylase in the blood were performed on this patient, the first record being made on the fourth day following admission to the hospital or on the sixth day of the patient's illness. Despite the initial determination of amylase well after the onset of the disease, the level

was distinctly elevated and the patient continued to be distressed. It may be presumed that an amylase determination taken immediately after hospital admission would have revealed a much higher value. Blood amylase levels of several thousand have been reported in cases of acute edematous pancreatitis, although it has been pointed out that there is little relationship between the amylase elevation and the severity of the disease. However, because it is not uncommon to find a sharp temporary rise and a rapid decline in the amylase level, the need for early and repeated determinations has been frequently emphasized.

Inasmuch as urine has a variable chemical reaction and composition, it is not surprising to find that urine amylase values fluctuate much more widely than do blood amylase values. Kidney damage and functional renal diurnal variation increase the bounds of this fluctuation. For these reasons, it has been found preferable to determine the blood amylase levels whenever possible.

Elevation of amylase values, when associated with a suggestive clinical syndrome, is most incriminating evidence of a pancreatic lesion. Patients with severe renal impairment are known to have a moderate elevation of blood amylase values, while those patients with hepatic impairment frequently have lowered amylase values; but confusion with these conditions is hardly to be expected. There is no disease other than acute pancreatitis that will cause so marked an amylase level rise if the determinations are made early in the course of the disease.

The impetus thus given to diagnostic precision is strikingly important when considered in relation to the manner of treatment. Although there is an extensive controversial literature concerning the surgical or nonsurgical treatment of pancreatitis, it may be said that there has been a notable recent trend in favor of the conservative point of view. Therefore, with the diagnosis of acute pancreatitis confirmed by laboratory data, it would seem advisable to postpone surgical treatment until such time as the biliary system can be carefully studied and the necessary procedures carried out.

It is felt that acute edematous pancreatitis is a frequently unrecognized cause of upper abdominal pain. A careful history and physical examination augmented by suitable amylase studies are of significant aid in the diagnosis of this disease.

METHOD EMPLOYED IN THE ESTIMATION OF AMYLASE

In the preparation of the reagents and the analytical procedure, the technique of Somogyi was closely followed. From his description I quote the following:

"Starch Paste.—We employ U.S.P. corn-starch or pure rice starch. The starch is washed as follows: 100 gm. of starch are suspended and frequently agitated for about an hour in 1 liter of approximately 0.01 N HCl. After sedimentation the acid is poured off and the starch is stirred up in 1 liter of approximately 0.05 per cent NaCl solution. After sedimentation and decantation washing with salt solution is repeated once more; then the starch is spread out and allowed to dry in air.

"A paste is prepared with approximately 15 gm. of washed starch per liter of water. The starch is thoroughly ground in a mortar with 50 c.c. of water, while 900 c.c. of water

are heated to boiling. The ground starch suspension is transferred into the hot water with vigorous agitation, 50 c.c. of water being used to rinse the mortar. After boiling for 0.5 to 1 minute (with agitation), the starch paste is heated in a water bath for 15 to 30 minutes. The mouth of the flask is kept covered by an inverted beaker during the heating period. Grinding exerts the favorable effect of keeping the starch in a well-dispersed state for a long time, whereas without the grinding it soon forms a heavy sediment.

"Acid NaCl solution.—This contains 10 gm. of NaCl and 3 c.c. of 0.1 N HCl per liter. The presence of acid is necessary for correction of the pH of blood serum or plasma which tends to rise above the upper limit of the optimum pH range (7.0 to 7.4) owing to the loss of CO_2 during the preparatory manipulations.

"Protein Precipitants.—A 5 per cent solution of $CuSO_4.5\ H_2O$ and a 6 per cent solution of sodium tungstate are used for deproteinization of plasma or serum."

The copper reagent, phosphomolybdic acid color reagent, and the standard glucose solutions are similar to the ones commonly used for the determination of reducing sugars.

ANALYTICAL PROCEDURE

"In a test tube of 14 to 16 mm. diameter 5 c.c. of the starch paste and 2 c.c. of the NaCl solution are mixed and immersed in a water bath of 40°. After a few minutes, when the fluid has assumed the temperature of the water bath, 1 c.c. of plasma or serum is added, the tube is stoppered and inverted a few times, and the mixture is incubated for 30 minutes. At this point the action of the enzyme is terminated in conjunction with the deproteinization of the reaction mixture. To this end 1 c.c. of the $CuSO_4$ solution is admixed, then 1 c.c. of the tungstate solution is added, and the mixture is well shaken and centrifuged. Since the copper salts inhibit but do not fully stop amylase action, these operations must be carried out without undue delay.

"The supernatant fluid, which is enzyme-free, is poured through a small filter paper. The filtrate, a 1:10 dilution of the serum, may be opalescent owing to the presence of unaltered starch, and is ready for the determination of its copper-reducing power." Somogyi completes the rest of the procedure by following the Shaffer-Hartmann technique. However, we have found that Benedict's modification of the standard Folin and Wu procedure is a simple and sensitive method for determining sugar.

The glucose content of the serum must be deducted from the total reducing substances, the difference then represents the reducing power (in terms of glucose) of the reaction products formed from starch by the amylase activity of 100 c.c. of serum. This figure expressed in milligrams per cent is denoted as amylase activity.

I am grateful to Dr. H. Stein for his technical assistance.

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HIGH INCIDENCE OF INFECTIVE STOOLS IN A SMALL OUTBREAK OF INFANTILE PARALYSIS*

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THIS paper is an account of work done on the stools of patients suffering from, or suspected of having, anterior poliomyelitis. The techniques used are based essentially on those developed by Trask, Vignec, and Paul,[1] and by Kramer and his co-workers,[2] but are modified somewhat to suit our purposes.

Stool specimens were received at the laboratory in 25 ml. sterile glass containers with metal screw tops, or in 250 ml. bacteriologic water bottles with ground-glass stoppers. No reagents were added to the specimens before they arrived at the laboratory. On receipt from 20 to 25 ml. were suspended in from 90 to 100 ml. of sterile distilled water in a wide-mouthed bottle with a ground-glass stopper. To this about 15 ml. of ethyl ether were added. The suspension was then shaken by hand for from five to ten minutes and placed in the refrigerator at a temperature of from 4° to 10° C. After twenty-four hours cultures were made on horse blood agar plates and incubated for twenty-four hours. If these plates showed only slight growth, and no streptococci were present, the suspension was evaporated under vacuum in a desiccator containing sulfuric acid to get rid of the ether. Three mice were inoculated intraperitoneally, each with 0.1 ml. of the suspension. If the mice survived twenty-four hours, it was considered safe to proceed with monkey inoculations. If the mice died, the specimen was treated again with ether, and the whole procedure was repeated. Usually two treatments were sufficient, although before the use of mouse controls two monkeys in the first series died of peritonitis from the lesions of which presumably pathogenic microorganisms were isolated.

Because of these deaths an attempt was made at first to get a better bactericidal agent by using vinyl ether, and also chloroform, but without success; and lastly by shaking the suspension from two to four hours in a shaking machine with ethyl ether. This last procedure sterilized the stools more effectively, but most of them proved to be toxic. The test mice consistently died within ten hours, and only slight bacterial growth was obtained from the peritoneum. Monkeys inoculated with such stools evidenced a severe and rapid toxemia with subnormal temperatures and evidence of paralytic ileus. Two of them died within twenty-four hours, and no microorganisms were isolated either from the peritoneum or from the heart's blood. Moreover, no monkeys inoculated with material treated in this manner developed poliomyelitis. We eventually returned to our original method with such success as will be pointed out subsequently.

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The stool inoculations in all cases were made intraperitoneally, from 5 to 20 ml. being given at an injection. Occasionally with two injections on successive days, as much as 30 ml. were given. Stool suspensions shaken only briefly with ether invariably settled out into layers. Upon injection the topmost, clearest layer and some of the middle, cloudier material were taken. The coarse sediment at the bottom of the bottle was not disturbed.

For monkey passage we used rather heavy 10 to 20 per cent suspensions of supposedly infected brain and spinal cord material, ground in the beginning with pulverized glass, but recently with alundum, and suspended in physiologic salt solution. The suspension was centrifuged at about 2,000 r.p.m. for ten minutes. Routinely 1.0 ml. of the supernatant was inoculated into the left frontal lobe, and 5.0 ml: were inoculated intraperitoneally. In each case, four immature mice were inoculated intracranially and intraperitoneally with the same suspensions. None of the mice at any time showed signs of disease.

The first group of patients consisted of four children with clinical poliomyelitis, and an adult believed to be suffering from a second attack of the disease. All were inmates of an institution and were convalescent. The cases were studied during April, 1939, through the courtesy of Dr. A. C. Silverman of the City Hospital, Syracuse, N. Y. The stool specimens were treated as described, with only slight shaking. Specimens from the four children proved infective for monkeys, and second or third passages were obtained. The animal inoculated with the specimen from the adult remained alive and well. Table I shows the relationships between the clinical disease and the collection of stools. It is of interest that in the case of S. C. a period of forty days elapsed between the probable onset of the disease and the collection of stool material. It is also of interest that all of these children, and another to be noted later, were under two years of age. All had flaccid paralysis of some degree, four in the extremities and one in the face.

The next group of cases studied was from a small outbreak near Germantown, N. Y., in August, 1939, investigated with the help of Dr. L. D. Carpenter. Stool specimens were obtained: one from a child with extensive paralysis in the first week of the disease, and another from a collateral contact who had had a brief febrile illness at about the same time but without paralysis. We were unable to produce poliomyelitis in monkeys, and one of three animals inoculated died of peritonitis; hemolytic streptococci were isolated.

With the cooperation of Dr. H. R. O'Brien and Dr. J. V. Anderson, a third group of cases from Cattaraugus County was studied in December, 1939, and January, 1940. It consisted of seven children in one family, two of them with paralytic anterior poliomyelitis, one recovered from a febrile illness without paralysis, and the other four not clinically affected; and two other individuals, one with residual paralysis six weeks after the onset and another with no paralysis one month following illness. The specimens from this group were obtained from three to six weeks after onset of the disease. They were treated by prolonged shaking with ether, and while no monkeys contracted clinical poliomyelitis, two died of peritonitis and paralytic ileus. No microorganisms could be cultured from either the peritoneum or heart's blood.

During August and September, 1939, a number of stool specimens were received in the laboratory from Buffalo, N. Y., and stored at an average temperature of –5° C. until they could be studied. On January 31, 1940, one of these specimens from an infant ill with paralytic poliomyelitis, received September 9, 1939, was selected and treated by the original method of shaking very briefly with ether. Cultures from the treated suspensions showed no growth. A monkey was inoculated and developed severe anterior poliomyelitis. The virus has been given three subsequent passages. It is of great interest that the virus remained virulent in this stool, frozen in the refrigerator for 124 days, or throughout the winter months.

TABLE I

PATIENT	PROBABLE DATE OF ONSET	STOOL SPECIMEN RECEIVED	MONKEYS INOCULATED FROM STOOL		PASSAGE MONKEYS INOCULATED		PASSAGE MONKEYS INOCULATED	
			ANIMAL NO. AND DATE	ANATOMIC FINDINGS	ANIMAL NO. AND DATE	ANATOMIC FINDINGS	ANIMAL NO. AND DATE	ANATOMIC FINDINGS
Syracuse								
A. Mc. F. 17 mo.	4/ 9/39	4/30/39	No. 83 5/ 3/39	Anterior poliomyelitis	No. 88 from 83 5/12/39	Meningoencephalitis	No. 101 from 83 and 88 12/12/39	Anterior poliomyelitis
N. W. M. 7 mo.	4/14/39	4/30/39	No. 76 5/ 3/39	Encephalitis	No. 98 from 76 11/21/39	Anterior poliomyelitis		
J. W. M. 14 mo.	4/ 9/39	4/30/39	No. 86 5/ 3/39	Anterior poliomyelitis	No. 91 from 86 5/11/39	Encephalitis. Light anterior poliomyelitis		
S. C. F. 22 mo.	"About" 4/ 2/39	5/12/39	No. 92 5/12/39	Anterior poliomyelitis	No. 100 from 92 6/ 6/39	Mild encephalitis	No. 96 from 92 and 100 12/22/39	Anterior poliomyelitis
Buffalo								
E. P. M. 9 mo.	8/23/39	8/31/39	No. 103 1/31/40	Anterior poliomyelitis	No. 113 from 103 3/ 8/40	Anterior poliomyelitis		

Note.—In patients A. Mc. and S. C. the material for final second passage was pooled as indicated, both for fear of losing the strain and to conserve monkeys. It was desired to establish a typical second passage only.

More complete clinical data on four of these cases are discussed by Dr. A. C. Silverman (Am. J. Pub. Health 21: 593, 1941).

Because passage monkeys sometimes fell ill with an acute disease showing involvement of the central nervous system but without flaccid paralysis, no stools were considered to have unequivocally contained the virus of anterior poliomyelitis until at least one typical second or further passage was obtained (see Table I). Thus, even though a monkey showed fever after from five to ten days, accompanied by tremor and other clinical signs of encephalitis, and even though such animals on autopsy showed meningitis with mononuclear cell exudate, perivascular accumulations and nerve cell destruction with neurono-

phagia in the brain stem or cortex but not in spinal cord, the evidence was considered insufficient. Further passages were made until a monkey developed, clinically and histopathologically, typical anterior poliomyelitis. As other authors[3, 4] have given detailed protocols of the disease in monkeys, it seems superfluous to do so here. Characteristic lesions consisted of neurone necrosis with neuronophagia in the anterior horns and usually elsewhere; perivascular cell accumulations usually of mononuclear cells, but occasionally with polymorphonuclear cells predominating; some degree of leptomeningitis, often of scattered distribution; occasionally small perivascular hemorrhages in the nervous tissue or meninges; signs of microglial activity and clasmatodendrosis of astrocytes about local lesions, and various degrees of Nissl's degeneration in ganglion cells. Lesions were observed in all major divisions of the spinal cord, the medulla, pons, mesencephalon, diencephalon, roof nuclei of the cerebellum, and the motor cortex, as well as in dorsal root ganglia and Gasserian ganglia. No lesions were seen in the thoracolumbar autonomic ganglia.

In many monkeys focal interstitial nephritis was observed. Similar lesions, however, have since been found in a monkey dying of unknown cause, and in two monkeys dying of bacillary dysentery.

All intracranial inoculations in monkeys *(Macacus rhesus)* were made into the left frontal lobe. All monkeys were sacrificed by light ether anesthesia and intracardiac air embolism. None were permitted to die spontaneously. Autopsy in each case was performed as soon as death supervened. In addition to the parts of the nervous system mentioned sections were taken routinely from all visceral organs, including the major divisions of the gastrointestinal tract, and from the peritoneal lymphatics. The viscera were fixed in Zenker-acetic solution and stained with hematoxylin and phloxine. Central nervous system tissue was fixed in Zenker-acetic solution, formalin ammonium bromide, and 70 per cent alcohol. Hematoxylin-phloxine and thionine stains and silver carbonate and gold-chloride sublimate impregnations were done in each case, and when it seemed indicated, scarlet red stain (for demonstration of fat) was made. In only one instance, however, was any considerable fatty change observed.

All autopsies were controlled by cultures and film preparations from the peritoneum, brain, and heart's blood on horse blood agar. In no case were any significant bacterial findings observed. All passage suspensions were controlled by culturing on horse blood agar, with no significant bacterial findings. All inoculations into monkeys were controlled by parallel inoculations into young Swiss mice. The mice were observed for one month. No instances of disease were noted. Virus strains, if one may use the term in this connection, from three different cases were put intracranially and intraperitoneally into Eastern cotton rats *(Sigmodon hispidus hispidus)*, but no successful passage was obtained.

Serial "faith" passages through six passage generations of cotton rats yielded no results. Cotton rats and young Swiss mice intoxicated with guanidine hydrochloride, diorthocresylphosphate, cadaverine hydrochloride, and ethylenediamine dihydrochloride, all failed to contract the disease. All were observed for a month following intracranial and intraperitoneal inoculation with tissue

containing virus. All chemical intoxications were maintained over a period of from ten days to two weeks, starting usually five days previous to exhibition of the virus. Three rats put on thyroxine injections all died during manipulation for taking temperatures before poliomyelitis would have been expected to develop.

It is worth noting here that many of our cotton rats died suddenly during or after very brief handling. We were unable to find a cause for death on autopsy, though it may be significant that immediately post mortem there was marked peristaltic activity observable through the intact abdominal wall.

SUMMARY AND CONCLUSIONS

Single random specimens of stool were obtained from a total of 17 cases of typical, abortive, or atypical suspected anterior poliomyelitis and inoculated into monkeys, with five "takes." Nine of these cases had clinical paralysis. Two were clinically abortive types. Two were atypical. Four were contacts without clinical signs or symptoms.

Eight stool specimens were treated by the method outlined, with shaking from five to ten minutes with ether. Of these eight cases, seven had paralysis. From five of the paralyzed cases, successful transfers of the disease into monkeys were obtained.

Nine stool specimens were treated by the method outlined, but the suspensions were shaken for two to four hours with ether. Of these nine cases, two were paralytic; none of the inoculated monkeys showed signs of poliomyelitis.

One of the infective stool specimens was collected as late as forty days after the probable onset of the disease.

One of the stool specimens kept at a temperature of −5° C. remained infective for 124 days after collection.

All stool and monkey cord passages were controlled by mouse inoculation.

All successful passages were from the stools of infants under two years of age, in four cases at least, on similar diets.

It does not appear to me that any deductive conclusions may be drawn with certainty at this stage of the investigations. However, the question under what conditions poliomyelitis virus will remain infective in stools seems to be of interest.

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BEHAVIOR OF THE RECIPIENT'S LEUCOCYTE COUNT FOLLOWING TRANSFUSION OF PRESERVED BLOOD*

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THE increasing use of preserved blood for transfusions has raised many questions regarding its effectiveness as compared to that of fresh blood. Among these questions is its relative value in agranulocytosis and in septicemia or other infectious conditions, both as regards the transferal of immune bodies and the leucocytes themselves. Although there is still a difference of opinion as to the effectiveness of any transfusion in combating an infection, the procedure is frequently used.

It has been shown that with a transfusion of fresh blood the transfused leucocytes disappear from the recipient's blood stream within two and one-half hours and that such a transfusion does not cause a significant increase in the leucocyte count.[1] Since the life of a normal leucocyte is from one to five days, and the number of these cells in the ordinary transfusion is relatively small, one could not expect a significant increase in the recipient's count. With preserved blood it has been shown that there is a gradual disintegration and reduction in the number of leucocytes during the period of storage, and it has been recommended that this blood not be used in patients with agranulocytosis.[2] The value of transfusions of preserved blood in infectious states has also been questioned, since the degenerative changes in the neutrophiles reduce their phagocytic activities.[3] On the other hand, it has been suggested that the products of disintegration may stimulate the formation of new cells in the recipient.[2]

In order to determine the effect of transfusion of preserved blood on the recipient's circulating leucocytes, counts were made before and after the giving of blood from the "blood bank." The age of the blood varied from one to twenty-five days, as is noted in the tables, and the preservatives used were either sodium citrate or a dextrose-citrate mixture, as described by DeGowin.[4] The patients selected for study were those without an acute infection or recent hemorrhage. No patient was studied during a postoperative period. In one group of 15 patients leucocyte counts were done at two-hour intervals following transfusion, and in a second group of 12 patients counts were done at twenty-four-hour intervals, so that information might be obtained on either an immediate or a delayed rise due to stimulation from the disintegration products. The results are shown in Tables I and II.

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TABLE I

LEUCOCYTE COUNTS AT TWO-HOUR INTERVALS

CASE	INITIAL COUNT		2 HOURS	4 HOURS	6 HOURS	8 HOURS	AGE OF BLOOD DAYS	DIAGNOSIS
1	7,300	Transfusion	7,050	10,050	8,200	7,050	22	Carcinoma of cervix
2	1,700		1,500	1,850	1,250	2,000	21	Aplastic anemia
3	2,400		1,950	1,650	2,450	1,900	1	Aplastic anemia
4	7,300		8,050	7,800	8,500	8,100	7	Peptic ulcer
5	4,000		4,300	5,100	5,600	4,550	6	Peptic ulcer
6	5,450		4,950	4,450	5,700	4,800	5	Peptic ulcer
7	5,250		5,650	6,700	5,050		25	Carcinoma of colon
8	8,300		8,500	7,100	7,500		25	Incomplete abortion
9	5,600		5,750	4,410	4,000		2	Aleucemic leucemia
10	10,650		10,750	11,200	10,800		3	Carcinoma of stomach
11	3,350		3,950	3,400	3,100		4	Aplastic anemia
12	12,950		13,680	13,900	11,950		4	Chronic cholecystitis
13	2,000		1,650	2,100			23	Aplastic anemia
14	11,300		11,500	10,800			19	Ulcerative colitis
15	10,050		10,650	10,800			2	Carcinoma of stomach

TABLE II

LEUCOCYTE COUNTS AT TWENTY-FOUR-HOUR INTERVALS

CASE	INITIAL COUNT		24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS	AGE OF BLOOD DAYS	DIAGNOSIS
1	8,500	Transfusion	9,600	9,400	10,100	11,200	9,000	1	Pernicious anemia
2	6,900		7,200	8,200	6,350	6,900	7,500	6	Peptic ulcer
3	7,200		7,000	7,800	6,350	7,750	6,250	6	Carcinoma of cervix
4	6,300		7,050	8,100	7,400	7,000	7,650	22	Carcinoma of cervix
5	10,200		10,550	9,350	8,050	8,400		3	Nutritional anemia
6	12,350		8,900	9,450	8,800	8,200		16	Carcinoma of stomach
7	5,250		5,850	4,250	3,600	3,700		25	Carcinoma of colon
8	3,600		3,800	3,400	3,900	3,700		2	Aleucemic leucemia
9	3,350		3,750	3,300	2,950	3,650		4	Aplastic anemia
10	11,300		9,650	10,050	10,350	10,050		19	Ulcerative colitis
11	8,450		9,450	8,660	7,900			10	Hypochromic anemia
12	8,300		7,600	7,000	7,900			25	Incomplete abortion

Examination of these tables reveals that there was no consistent change in the leucocyte count as a result of the transfusion. An elevation in the count occurred in some cases, but this was not constant; in other cases a reduction in the count was noted. These variations are no greater than can be accounted for by the unavoidable error in the method, the diurnal variations, or the rhythmic variations in the circulating leucocytes.[5-7] The results are in accord with what one would expect from our knowledge of the life of the leucocytes and their behavior during storage. The age of the blood made no difference in the results nor did the type of case to which it was given. In aplastic anemia one could expect no latent rise from the stimulation of disintegration products, and although such a reaction might occur in cases with normal bone marrow, it was not encountered in this group of cases.

Transfusions of preserved human blood produce no constant or significant changes in the leucocyte count of the recipient.

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THE EFFECTS OF DECREASED TEMPERATURE ON THE ACTIVITY OF INTACT MUSCLE*

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SINCE muscular contraction involves intrinsic chemical and physical changes, the influence which a fall in temperature has on muscular activity may be anticipated by studying the effects which temperature changes have on the processes involved in muscular contraction. However, a mere statement of the qualitative effects which temperature changes have on muscular contraction is inadequate for a complete explanation of poor performance, and also muscle injury, experienced by individuals who are called on to exercise strenuously.

In order to have a clearer picture of the effects of lowering the temperature of a muscle on its activity, the experiment herein reported was performed. To make the experimental data parallel the conditions as they actually exist, the activity of the intact gastrocnemius muscle and its antagonist when exposed to cold, was studied. In order to determine the effects of lowering the temperature of the muscle on its activity, the form curve of a normal muscle was compared with that of the same muscle after it has been cooled for various periods of time.

The gastrocnemius muscle was caused to contract by applying submaximal break induction shocks to it. This was accomplished by attaching one pole of the inductorium to an electrode placed over the medial motor point of the muscle, and the other pole to an electrode placed on the thigh, over an indifferent area. A foot switch was employed in the primary circuit so that the experimenter's hands were free to manipulate the recording apparatus.

The subjects were placed in a prone position on a table with the feet well over the edge. A cord was attached to the toe of the subject's shoe, thence over a pulley under the table to a recording stylus suspended from a rubber band. The rubber band furnished the means by which uniform tension was maintained on the muscle throughout the experiment. A signal magnet, placed in the primary circuit, was superposed above the recording stylus. A 100 double vibration electrically driven tuning fork was placed between the recording stylus and the signal magnet. The record was made on an extension kymograph which

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was operated manually. Obviously, the records were recorded upside down, since a contraction of the muscle caused an extension of the foot, thus pulling the recording stylus down.

The procedure in securing the data was first, to make control records from the muscle, and then pack the leg in ice. After five, ten, and twenty minutes of cooling, additional records were made. Fifteen to twenty tracings were secured under each condition studied.

By means of ordinates the response of the muscle was divided into its latent period, periods of contraction and relaxation. The length of each period was determined by counting the number of tuning fork vibrations between the ordinates.

THE DATA

Data were collected from 12 individuals. The values given in Table I are the means of no less than 15 responses per period for each individual.

TABLE I

A COMPARISON OF THE EFFECT OF COOLING ON THE PERIODS OF RESPONSE OF THE INTACT GASTROCNEMIUS MUSCLE

	LATENT TIME	PER CENT INCREASE	CONTRACTION TIME	PER CENT INCREASE	RELAXATION TIME	PER CENT INCREASE
Normal	0.024 ± 0.002		0.089 ± 0.006		0.267 ± 0.034	
Cooling 5 min.	0.027 ± 0.002	12.5	0.108 ± 0.012	21	0.432 ± 0.035	62
Cooling 10 min.	0.030 ± 0.002	25	0.130 ± 0.015	46	0.574 ± 0.045	115
Cooling 20 min.	0.033 ± 0.002	37.5	0.162 ± 0.024	82	0.726 ± 0.061	172

Although the well-known facts relative to the effects of cooling muscle are demonstrated by the experiment, the phase of activity in which we are most concerned in this investigation is the period of relaxation. After five minutes of cooling the relaxation time is increased 62 per cent, while the period of contraction and the latent time are increased 21 per cent and 12.5 per cent, respectively. This means that relaxation is affected three times as much as contraction and five times as much as the latency of the muscle. Ten minutes of cooling increases the period of relaxation 115 per cent, while the contraction time is increased only 46 per cent and the latency 24 per cent. Under this condition, relaxation is increased 2.5 times as much as contraction and 4.6 times that of the latency. After twenty minutes of cooling the situation is similar. Relaxation is increased 172 per cent, contraction 82 per cent, and latency 37.5 per cent. Under this condition, relaxation is increased 2.1 times as much as contraction and 4.6 times as much as latency. In view of these facts one is justified in emphasizing that cooling a muscle not only affects the relaxation more than any other period of its activity, but also slows this period from two to three times as much as the contraction.

The effect of cooling on muscular relaxation is especially important to athletes who participate in sports, such as sprinting, during which the muscles perform explosive movements. As far as the latent and contraction periods are concerned, the warming-up of a muscle may be adequate, but from the point of view of relaxation at that stage it is totally unprepared for explosive action. Such a condition makes for poor performance, since a succeeding contraction cannot occur until the muscle relaxes from the preceding contraction.

A complete warm-up also is important from the standpoint of muscle injury, since when the driving muscles contract, their antagonists must relax in a synergistic fashion. Injury to an improperly warmed-up antagonist is not uncommon. Such injuries can only be prevented by sufficient preliminary exercises to reduce the relaxation time of an antagonist commensurate with the reduction in contraction time.

It is also worthy of note that muscle injury may occur if a properly warmed-up muscle is exposed to a cold environment. Such occurrences as these can be prevented only by protection from cold. In fact, many sprinters, running from a protected environment into a cold draft or breeze, have been observed to have experienced muscle injury.

SUMMARY AND CONCLUSIONS

An investigation of the effect of cooling intact muscle shows that the lowering of the temperature increases the relaxation time two to three times as much as the contraction time of the muscle. Inadequate relaxation due to an improper warm-up is sufficient to explain poor performance in explosive bouts of exercise such as sprinting. Also, injury to muscles antagonistic to driving muscles is explained on the basis of a prolonged relaxation time, due to either an inadequate warm-up, a cold environment, or a sudden change in the environment.

A STUDY OF THE THERAPEUTIC EFFECT OF SULFAPYRIDINE IN PNEUMOCOCCUS INFECTED MICE IN ATMOSPHERES OF VARYING OXYGEN TENSION*

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INTRODUCTION

INVESTIGATIONS on the mechanism of the action of sulfanilamide and its derivatives in vitro have led to the theory that an oxidation product of the drug is responsible for the bacteriostasis noted (Mayer;[1] Ottenberg and Fox;[2] Fox, Cline, and Ottenberg[3]). As a corollary to this theory the bacteriostatic action of the drug should be lessened under anaerobic conditions of culture. Swift, Lancefield, and Goodner[4] found that with 6 strains of hemolytic streptococci, if removal of oxygen and reduction are complete before inoculation of the media with the organisms, bacteriostasis by sulfanilamide is prevented. Shinn, Main, and Mellon[5] demonstrated that reduction of the oxygen in superambient air of broth cultures of type I pneumococcus reduced or prevented bacteriostasis by sulfanilamide. This inhibition of bacteriostasis was also noted under complete anaerobic conditions. When, however, the superambient air contained 0.04 per cent oxygen, this inhibition effect was absent. Fox,[6] studying

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this same phenomenon, concludes that complete initial deprivation of oxygen interferes with bacteriostasis and increased oxygen availability magnifies bacteriostasis. Unpublished studies by Fox[7] on the effect of oxygen deprivation on the action of sulfapyridine on pneumococci demonstrate that the action of the drug is lessened under anaerobiosis. This is in disagreement with Long,[8] who could not demonstrate this effect. Long's report shows that in both aerobic and anaerobic cultures he obtained sterility. Using streptococcus as the test organism, Long finds a lessened bacteriostatic action of the drug under anaerobic conditions.

On the basis of this work it was felt that perhaps a reduction of the oxygen saturation in the blood of mice would similarly interfere with the action of sulfapyridine on pneumococcus septicemia. Conversely, an increase in oxygen saturation should enhance the bacteriostasis.

EXPERIMENT 1

Infecting dose: 100 M.L.D.

OXYGEN PER CENT	NO. OF MICE	TREATMENT	DEATH IN DAYS									NO. DIED	NO. SURVIVED
			1	2	3	4	5	6	7	8	9		
20.8	9	Infected (control)	9	0	0	0	0	0	0	0	0	9	0
20.8	15	Infected and drug	1	0	1	0	1	4	3	2	2	14	1
8	15	Infected and drug	3	2	1	1	1	2	2	1	0	13	2
60	13	Infected and drug	1	1	1	2	0	3	1	3	0	12	1

EXPERIMENT 2

Infecting dose: 1,000 M.L.D.

OXYGEN PER CENT	NO. OF MICE	TREATMENT	DEATH IN DAYS									NO. DIED	NO. SURVIVED
			1	2	3	4	5	6	7	8	9		
20.8	17	Infected (control)	17	0	0	0	0	0	0	0	0	17	0
20.8	17	Infected and drug	0	1	0	3	4	4	2	2	1	17	0
8	17	Infected and drug	4	1	2	1	4	3	2			17	0
60	17	Infected and drug	1	1	2	3	5	1	3	1		17	0

METHODS

For these experiments we used a mucoid type I pneumococcus which was passed through rabbits once a month and kept in rabbit's blood under vaseline seal. For infection a subculture was made using 0.5 c.c. of this stock culture. The infecting volume was 0.3 c.c. of the given dilution (in broth) of a five-hour subculture, injected intraperitoneally.

The drug sulfapyridine was suspended in 10 per cent gum acacia and fed twice daily, giving 30 mg. of the drug per day for six days and 15 mg. daily for the seventh and eighth days. The given atmospheres were continued for twenty-four hours after the last drug dose, and the experiment was considered complete twenty-four hours after discontinuation of the gases. In all cases, cultures from heart blood were taken at autopsy and the organisms were recovered. In each of the experiments a group of normal mice was put in with the low oxygen group to control deaths due possibly to the low oxygen.

To control the factor of the effect of varying the oxygen atmosphere on pneumococcus septicemia, three groups of 15 mice each were infected with 100 M.L.D. of the culture and subjected to 8 per cent oxygen, 60 per cent oxygen, and room air (20.8 per cent), respectively. In all groups the mice died between fourteen and twenty-four hours. The distribution of deaths was approximately the same in all three groups.

Varying the oxygen concentration of the atmosphere did not appear to affect the mortality rate.

Five additional similar experiments were run in which a total of 225 mice were exposed to low and high oxygen percentages, and the conditions in Experiments 1 and 2 duplicated. Since the results in these experiments were confirmatory of the foregoing data, a detailed presentation is not given.

CONCLUSIONS

In cultures of pneumococci anaerobic conditions have been reported to prevent the bacteriostatic action of sulfapyridine.

Pneumococcus-infected mice were treated with sulfapyridine in atmospheres of low oxygen tension (8 per cent), air and high oxygen tension (60 per cent) without significant effect on the mortality rate. Varying the oxygen tension of the atmosphere does not appear to modify the effectiveness of sulfapyridine in pneumococcus septicemia.

It is acknowledged that these results do not apply to pneumonia in which the oxygen tension of the capillary blood may fall to far lower levels.

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GOLD COLLOID AND COLLOIDS OF OTHER HEAVY METALS IN THE TREATMENT OF RHEUMATOID ARTHRITIS*

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THE rapidly accumulating literature on the use of gold salts in rheumatoid arthritis is in apparent harmony on the following two points: (1) Gold in the form of its salts is perhaps the most efficient agent known in the treatment of rheumatoid arthritis. (2) The toxic or untoward reactions so frequently encountered are a serious drawback to the use of this drug. The clinical results I[1] obtained with gold sodium thiomalate in a recent investigation undertaken with the purpose in view of reducing the frequency and severity of toxic phenomena served to strengthen these views.

With the two enumerated points in mind, therefore, I was induced to search for some similar or related agent, or agents, that might incorporate all the beneficial therapeutic qualities of gold salts without their serious toxic effects. It was also my aim to ascertain whether the beneficial attributes of gold were due to something inherent in the gold radical alone, regardless of chemical affinity; or whether these attributes were likewise demonstrable in the radicals of other heavy metals such as platinum, bismuth, etc.

Therapeutic research on the use of gold for some unascertained reason appears to have been limited to the salt. Review of the literature failed to discover any published reports on the treatment of rheumatoid arthritis with the colloid of gold or of other heavy metals. Several authors have reported using "colloidal" gold, but on closer investigation it was found that the term "colloid" had been used somewhat indiscriminately.

As is well known, the inorganic salts of gold are very reactive chemically. Most of them decompose readily, with the liberation of metallic gold, while even those which are generally regarded as fairly stable are quite reactive. As an example, a dilute solution of gold chloride, $AuCl_3$, decomposes on heating, while on exposure to direct sunlight metallic gold is precipitated and hydrogen peroxide is formed in the solution. The chemical reactivity of the somewhat more stable aurichloric acid, $HAuCl_4$, was observed by Boyle in 1663, who found that it would color the skin, nails, or ivory a purplish-red tint when exposed to light. This, of course, immediately calls to mind the staining of the skin experienced with solutions of silver nitrate. Even gold sodium thiosulfate, which has achieved a definite measure of recognition in the treatment of lupus erythematosus, is unstable, its solutions becoming yellow and decomposing on standing.

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The colloid solutions for this study were especially prepared by Crookes Laboratories, Inc., New York, N. Y.

It seems obvious, therefore, that in gold we have a highly reactive element, and that this reactivity may in part be responsible for the considerable toxicity of its salts. The thiomalate with which my associates and I have had considerable experience is an organic salt of gold which does not seem to be quite so reactive as the thiosulfate, and which in consequence can be, and is, used in considerably larger doses. However, even the thiomalate must be administered with considerable caution.

In view of this toxicity it seemed desirable to explore the possibility of introducing gold in a form which was less reactive chemically and which, in consequence, might permit a more sustained, effective, and less toxic form of therapy. Colloidal gold appeared to offer definite possibilities.

Within recent times reports on the use of dilute solutions of colloidal gold in the treatment of malignancy have been published,[2-8] and although the results obtained have in no way been conclusive, there are indications that even in the dilute solutions employed colloidal gold exerts a definite analgesic action.

Although metallic gold itself is a very stable metal, being unaffected by air and water and many ordinary laboratory reagents and solvents, it is not absolutely insoluble. When exhibited as a colloid, this element consists of innumerable particles of metallic gold. It seemed, therefore, particularly in this extremely subdivided form, that when injected in the body it would act in a manner similar to that ascribed to colloidal silver.

It will be remembered that when colloidal silver was made available commercially, new therapeutic fields were created for this element, which, in the form of its soluble salts, was far too reactive and toxic for use in more than very dilute solutions, except where a definitely caustic action was desired.

New and Nonofficial Remedies, referring to colloidal silver preparations, points out that the silver in these colloidal suspensions does not exist to any great extent in the free ionic state, and, therefore, does not precipitate chlorides and proteins. As a result it is noncorrosive and nonirritant, yet retains a definite degree of antiseptic action. This silver action is not proportionate to the total silver content of the colloidal silver preparation and varies with the different colloidal silver compounds. The antiseptic action of these colloids is undoubtedly due to the liberation of continuous, very low concentrations of silver ions.

Weiser[8] states that the more finely subdivided the silver, the stronger its bactericidal action. By analogy, therefore, colloidal gold, because it is almost devoid of gold ions and consequently reduced of its chemical reactivity, should not react immediately with the albumen, proteins, and salts which are found in the body, thereby permitting intramuscular administration without any immediate local irritation or general reaction.

My experience indicates this to be true. Furthermore, the billions of tiny particles of metallic gold should conceivably act as depots for the formation of gold ions, to which part of the action of gold salts may be ascribed, resulting in a more sustained action of the colloid but of a somewhat lower intensity than that obtained with gold salts.

There is another factor which might, theoretically, impart to a colloid of gold a beneficial action probably not associated with gold salts. It is well

known, for instance, that the injection of any colloid into the blood stream is attended by a certain amount of shock similar to that which usually follows the injection of a nonspecific protein. Thus, when using colloidal gold, with the therapeutic action of the gold itself, one is able to combine a mild nonspecific reaction.

Colloidal platinum (at.wt. 195.2) and colloidal bismuth (at.wt. 209.0) were included with gold in this study, since these metals have atomic weights approximating that of gold (197.2), and since it was thought possible that some common heavy metal action might be demonstrable therapeutically. Selenium, though not a heavy metal, because of its vaunted analgesic action in malignancies, which seemed to correspond in some way with this action of colloidal gold, was also included in this study. Selenium, however, together with platinum and bismuth, gave insufficient promise of therapeutic usefulness and was discontinued early in the study. Lead and some of the other metals in the higher scale of atomic weights were discarded as probably being too toxic.

It was felt that at least a period of two years from the initiation of this work should elapse before a more or less accurate evaluation of the therapeutic efficacy of the agents employed could be attempted with any degree of accuracy.

The duration of treatment in each individual case varied from three to twelve months. As with the salt, treatment consisted of three series of injections. Each series comprised 1 Gm. of metallic gold, an interval of six weeks being interposed between each series. An average of approximately 1 Gm. of gold was administered in each individual case.

In the beginning of this work a 0.1 per cent solution of colloidal gold was injected in 5 c.c. doses intragluteally. With this relatively dilute solution the actual amount of gold administered was very small, amounting to no more than 5 mg. (0.005). In spite of the relatively large quantity of solution injected, however, it was felt that larger doses of metallic gold, equal to those used by other investigators and myself, should be employed if an actual comparison of therapeutic results was to be obtained. Therefore, a second and more concentrated 1 per cent solution was prepared in which 2.5 c.c. of the suspension corresponded to 0.025 Gm. of metallic gold. This more concentrated solution in a number of cases resulted in an unsightly coppery discoloration at the point of injection, and for this reason had to be administered intragluteally. In both these suspensions the gold was "stabilized" with an emulsoid colloid containing 0.3 per cent tricresol.

For the sake of this therapeutic comparison it was deemed unfortunate that larger amounts of gold corresponding to the higher dosage brackets (0.05 to 0.1 Gm.) employed by Forestier and other investigators were not used at this time. In justification it must be remembered that in this study of the therapeutic effects of colloidal gold in rheumatoid arthritis we were treading, more or less, on virgin soil, and for that reason, had to exert a greater amount of caution. In passing, it must be mentioned, however, that far larger doses than those here employed have been used in the treatment of carcinoma and other malignancies.

International Atomic Weights

Au	Pt	Bi	Ir	Pb	Hg	Ra	Th	(Se)
197.2	195.2	209.0	193.1	207.2	200.6	226.0	232.15	(79.2)

Metals Administered

Total No. Patients Treated	Au	Pt	Bi	Se
50	36	4	4	6

Results of Colloidal Gold Therapy in Rheumatoid Arthritis

Total No. Cases Treated	Unimproved	Slight	Moderate	Marked	Uncertain
36	9	4	2	19	2

Fifty patients were injected with the above-mentioned colloids. Of these, 36* received gold, 4 platinum, 4 bismuth, and 6 selenium. Practically all these patients had received some previous form of therapy with unsatisfactory results. Two had been treated with gold salts in the form of gold sodium thiomalate intramuscularly, and two had had gold sodium thiosulfate with equally disappointing results. Among the series treated with the gold colloid were included two cases of generalized fibrositis that for years had failed to respond to any other form of therapy. The duration of the disease varied from three months to fifteen years. As to the severity or the grade of involvement of the rheumatic process, it might be said that the patients conformed to the average run of chronic cases currently seen in a large metropolitan clinic. The pathology varied from marked deformities as found in patients bedridden for a number of years, to those milder forms merely showing periarticular soft tissue changes. The usual laboratory procedures essential to the use of gold salts, i.e., sedimentation rate, complete blood studies, and urinalysis, were carried out before, during, and after initiation of therapy. Of 36 patients treated with gold colloid, 19, or 52.7 per cent, showed a definite improvement, while 9, or 25 per cent, failed to show any degree of amelioration. The two patients suffering from fibrositis demonstrated a quick and gratifying response.

Statistics in over 1,600 persons treated with gold salts show that there is an average marked improvement of about 54 per cent and a total lack of improvement in approximately 13 per cent. Considering the relatively small number of cases presented in this preliminary report, the difficulty of drawing valid conclusions as to comparative therapeutic value of gold salts and the colloid of gold is apparent, and any inferences derived from this study must have, therefore, only a relative value.

DISCUSSION

Of the metallic colloids employed, gold, because of its apparent therapeutic superiority over the other afore-mentioned metals, received predominant attention.

In drawing a differential comparison between gold salts and the colloid of gold, the following three criteria appear of outstanding importance: (1) therapeutic efficiency, (2) toxicity, (3) biological action. What the results are with the use of gold salts is common knowledge and repetition appears unnecessary.

*Seven of these patients were treated in the arthritis clinics of the Brooklyn and Post Graduate Hospitals, Doctors G. E. Anderson and Edward Hartung, respectively, in charge.

(1) *Therapeutic Efficiency of Colloidal Gold.*—In keeping with a definite subjective improvement in the majority of persons treated, there was a tendency toward regression of those signs which together comprise the rheumatoid picture. As in the case of gold salts, this regression consisted of reduced pain and periarticular swelling as well as of increased function of the parts involved. It was my impression, however, that the therapeutic response was lacking the quick, dramatic character often observed with gold salts.

(2) *Toxicity.*—In contrast to the highly toxic nature of gold salts, as occasioned by their ready solubility and intimate chemical action on the body tissues, thereby detracting from their value as routine therapeutic agents, the colloid, in the individual and total doses already mentioned, gave little evidence of such toxicity. This almost complete lack of toxicity is in conformity with the physicochemical structure of all colloids, which, as has already been mentioned, are, chemically, relatively inert substances and therefore exert little, if any, destructive action on the body tissues. With the colloid, for instance, there were none of those toxic, and sometimes alarming, clinical manifestations so frequently obtained with gold salts. In the entire series treated, one patient developed an enlargement of the axillary lymph glands. This patient, upon investigation, had been previously treated with gold salts. This glandular reaction has been found to be not an uncommon sequel to the use of the salt as well.

(3) *Biological Action.*—Biological action as determined by the sedimentation rate, complete blood studies, and urinalysis are as follows: As with gold salts, there was a parallelism between the drop in sedimentation rate and clinical improvement. This drop, however, in keeping with the slower therapeutic response, was definitely delayed.

At no time did any marked or abnormal changes occur in the urinary picture. If one realizes that a good part of any heavy metal, such as gold or mercury, is eliminated through the kidneys, one is again struck with the basically inert nature of the metallic colloids.

The essentially different nature of gold salts as compared with colloidal gold has been further emphasized by bacteriostatic tests with the sera of patients treated with both these substances. For instance, the sera of patients treated with gold salts showed a very active bacteriostatic power even after as few as three injections. In contrast, the colloid-treated serum utterly failed to retard the growth of organisms. These experiments conducted independently by Hartung and Sabin were in complete accord on the results obtained. This, in my opinion, is not necessarily proof of the inefficacy of the colloid. The very nature of its action, i.e., slower ionization, greatly diminished chemical reactivity, etc., would eliminate any attributes of great bacteriostatic power, especially after merely three or four injections.

MECHANISM OF COLLOID THERAPY IN RHEUMATOID ARTHRITIS

Studies of the reticulo-endothelial system in the deposition of colloidal and particulate matter in articular cavities furnish us with some very interesting data. Kuhns and Weatherford[10] describe experiments dealing with the deposition of trypan blue in the reticulo-endothelial system in and about the joints of the albino rats. These authors concluded that the colloidal and particulate matter used in their experiments is carried by the blood stream from various

parts of the body and stored in the histiocytes of the synovial membrane. Some amounts are also stored in the bone marrow, intermuscular septi and articular fat pads. Inflammation of the articular tissues tends to increase the amount of particulate matter deposited. The inferences which follow these experiments are that stimulation of the reticulo-endothelial system might be effected by the deposition of colloidal matter and that this stimulation should prove a definite advance in the treatment of articular diseases.

It is my belief that by the use of colloidal gold we are employing a substance which, as in the case of trypan blue, is deposited in various parts of the body, particularly the reticulo-endothelial system. The deposition of finely divided particles of gold in the histiocytes of the synovial membrane should result in the removal of products of inflammation, increase the defense mechanism in the synovial membrane, and likewise produce a stimulation of the general reticulo-endothelial system.[11]

CONCLUSION

The conclusions derived from a review of the results obtained with gold salts and with the colloid of gold are as follows: Gold salts are more active therapeutically and likewise are more toxic. In view of the known clinical and biological action manifested by gold salts in the more severe forms of rheumatoid arthritis, they are probably superior to the colloid. On the other hand, the encouraging and apparently superior results obtained with the colloid, as compared with some of the older agents, especially in the less severe forms of rheumatoid arthritis and in fibrositis, are in support of the opinion that a therapeutic niche lying somewhere between the salt and some of these older and less spectacular agents should be conceded the colloid.

These results, of course, are not incontrovertible, but in view of the encouraging results obtained thus far by this study, and in view of the scarcity of actually desirable therapeutic agents in rheumatoid arthritis and other forms of infectious arthritis, colloid gold therapy should be given the opportunity of continued unbiased investigation. It might also appear that this form of therapy might be especially desirable in the treatment of generalized fibrositis with an infectious background, in the arthritides of known infectious origin, or in the incipient forms of rheumatoid arthritis.

SUMMARY

1. In view of the toxicity of gold salts in the treatment of rheumatoid arthritis which renders this type of therapy a definitely hazardous one, the therapeutic possibilities of some of the less soluble forms of gold were investigated. Gold in colloid form was chosen because of certain definite chemical considerations.

2. With the purpose in view of determining whether the beneficial attributes of gold were (something) inherent in the gold radical alone or whether these attributes were also demonstrable in the radicals of other heavy metals, platinum and bismuth were also employed.

3. Selenium, though not a heavy metal, because of its vaunted analgesic action in malignancies, was likewise used.

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At no time did any marked or abnormal changes occur in the urinary picture. If one realizes that a good part of any heavy metal, such as gold or mercury, is eliminated through the kidneys, one is again struck with the basically inert nature of the metallic colloids.

The essentially different nature of gold salts as compared with colloidal gold has been further emphasized by bacteriostatic tests with the sera of patients treated with both these substances. For instance, the sera of patients treated with gold salts showed a very active bacteriostatic power even after as few as three injections. In contrast, the colloid-treated serum utterly failed to retard the growth of organisms. These experiments conducted independently by Hartung and Sabin were in complete accord on the results obtained. This, in my opinion, is not necessarily proof of the inefficacy of the colloid. The very nature of its action, i.e., slower ionization, greatly diminished chemical reactivity, etc., would eliminate any attributes of great bacteriostatic power, especially after merely three or four injections.

MECHANISM OF COLLOID THERAPY IN RHEUMATOID ARTHRITIS

Studies of the reticulo-endothelial system in the deposition of colloidal and particulate matter in articular cavities furnish us with some very interesting data. Kuhns and Weatherford[10] describe experiments dealing with the deposition of trypan blue in the reticulo-endothelial system in and about the joints of the albino rats. These authors concluded that the colloidal and particulate matter used in their experiments is carried by the blood stream from various

parts of the body and stored in the histiocytes of the synovial membrane. Some amounts are also stored in the bone marrow, intermuscular septi and articular fat pads. Inflammation of the articular tissues tends to increase the amount of particulate matter deposited. The inferences which follow these experiments are that stimulation of the reticulo-endothelial system might be effected by the deposition of colloidal matter and that this stimulation should prove a definite advance in the treatment of articular diseases.

It is my belief that by the use of colloidal gold we are employing a substance which, as in the case of trypan blue, is deposited in various parts of the body, particularly the reticulo-endothelial system. The deposition of finely divided particles of gold in the histiocytes of the synovial membrane should result in the removal of products of inflammation, increase the defense mechanism in the synovial membrane, and likewise produce a stimulation of the general reticulo-endothelial system.[11]

CONCLUSION

The conclusions derived from a review of the results obtained with gold salts and with the colloid of gold are as follows: Gold salts are more active therapeutically and likewise are more toxic. In view of the known clinical and biological action manifested by gold salts in the more severe forms of rheumatoid arthritis, they are probably superior to the colloid. On the other hand, the encouraging and apparently superior results obtained with the colloid, as compared with some of the older agents, especially in the less severe forms of rheumatoid arthritis and in fibrositis, are in support of the opinion that a therapeutic niche lying somewhere between the salt and some of these older and less spectacular agents should be conceded the colloid.

These results, of course, are not incontrovertible, but in view of the encouraging results obtained thus far by this study, and in view of the scarcity of actually desirable therapeutic agents in rheumatoid arthritis and other forms of infectious arthritis, colloid gold therapy should be given the opportunity of continued unbiased investigation. It might also appear that this form of therapy might be especially desirable in the treatment of generalized fibrositis with an infectious background, in the arthritides of known infectious origin, or in the incipient forms of rheumatoid arthritis.

SUMMARY

1. In view of the toxicity of gold salts in the treatment of rheumatoid arthritis which renders this type of therapy a definitely hazardous one, the therapeutic possibilities of some of the less soluble forms of gold were investigated. Gold in colloid form was chosen because of certain definite chemical considerations.

2. With the purpose in view of determining whether the beneficial attributes of gold were (something) inherent in the gold radical alone or whether these attributes were also demonstrable in the radicals of other heavy metals, platinum and bismuth were also employed.

3. Selenium, though not a heavy metal, because of its vaunted analgesic action in malignancies, was likewise used.

4. Platinum, bismuth, and selenium gave insufficient promise of therapeutic usefulness early in this clinical study and were, therefore, discarded.

5. Thirty-six patients in all were treated with the gold colloid over a period of two years. The colloid was given intramuscularly in individual and total doses equal to those I employed in a previous study with gold salts.

6. As a result of the present findings it is my opinion that in the gold colloid we are dealing with a more or less chemically inert form of gold, which, in contrast to gold salts, is only slightly soluble when injected in the body, and hence far less toxic.

7. The therapeutic results obtained with gold colloid may be partially explained on the basis of distribution of innumerable metallic particles throughout the reticulo-endothelial system, particularly in the deposition of these particles in the histiocytes of the synovial membrane, with the gradual liberation of gold ions and their consequent action on the body tissues.

8. No dramatic therapeutic qualities are espoused for colloidal gold in rheumatoid arthritis. In view of the beneficial response obtained in the present series, and because of the marked decrease in toxicity, it appears, however, that gold in colloid form is an agent worthy of further study and consideration for treatment of rheumatoid arthritis and generalized fibrositis with a toxic or infectious background.

I am indebted to E. A. H. King of the Crookes Laboratories, Inc., for his technical advice in preparing this paper.

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31 MONROE PLACE

THE RELATION OF THE NASOPHARYNX TO ULCERATIVE COLITIS*

Preliminary Report

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A VOLUMINOUS literature has developed in the field of ulcerative colitis as to etiology and treatment. It is with full knowledge of these facts that we add our preliminary report of treatment of this condition.

Nonspecific ulcerative colitis is a chronic disease of unknown etiology, characterized by exacerbations and remissions, with symptomatology of bloody diarrhea and loss of weight, with proctoscopic findings of an edematous, granular, easily bleeding mucosa with ulcerations, either punctate or serpiginous; with blood findings of a secondary anemia, x-ray findings of the colon showing loss of haustrations and narrowing of the lumen. In these cases bacteriologic studies do not reveal any *amoeba histolytica, B. dysenteriae,* or diplostreptococci.

Our studies began four years ago when it became apparent to one of us (S.W.) as well as to others, notably Kunstler, Bargen, and Darrow, that exacerbations in ulcerative colitis were preceded very frequently by upper respiratory infections. We then began to study cultures taken from the nose, throat, and rectosigmoid mucosa of ulcerative colitis cases, and collected a series of 15 cases between 1934 and 1938.

The ages of these patients varied from 13 to 45 years, with typical histories of from five weeks' to eleven years' duration. Most of these patients had been under medical care before coming under our observation. The symptomatology included abdominal cramps, bloody diarrhea, loss of weight, weakness, and anemia. The proctoscopic findings showed granular mucosa, freely bleeding and ulcerated. Bacteriologic studies of the nose and throat revealed *staphylococcus albus,* 6 cases; hemolytic streptococcus, 4 cases; streptococci (nonhemolytic), 2 cases; *Streptococci viridans,* 1 case; *Staphylococci hemolyticus,* 1 case; *Micrococcus catarrhalis,* 1 case. Bacteriologic cultures taken directly from the mucosa of the rectosigmoid revealed *B. coli,* 9 cases; *Staphylococcus albus,* 5 cases; streptococci (nonhemolytic), 3 cases; *B. coli hemolyticus,* 2 cases; *Streptococci hemolyticus,* 1 case.

All patients except one were treated with a combined vaccine; this patient was given only a vaccine prepared from smears taken from the rectosigmoid. Vaccine from the nose and throat was added in this latter case, and after three injections of the combined vaccine, the bowel movements became formed and blood disappeared from the stools. These 15 patients have been well for a period ranging from nineteen months to four years.

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Vaccines were prepared only from those organisms which were present simultaneously in the nose, throat, and rectosigmoid cultures. These vaccines were standardized to contain 1,000,000,000 organisms per cubic centimeter. Three dilutions were used, namely, 1:1, 1:10, and 1:100. We injected these vaccines three times a week in increasing doses from 0.1 c.c. to 1 c.c. for a total of 15 or more injections. Each week the three dilutions were used; the 1:100 the first day, the 1:10 the next injection, and the 1:1 dilution the last injection of that week. Each injection was increased by 0.1 c.c. each week.

The following is a case reported in detail because of the marked severity of the symptoms which improved after the combined vaccine therapy was instituted:

A young boy, 13 years of age, was admitted to the Polyclinic Hospital January 3, 1938, for the first time. For five weeks prior to his admission he had been having daily eight to ten loose stools, containing large amounts of bright red blood, severe generalized abdominal cramps, progressive loss of weight, and increased pallor. He also gave a history of staphylococcus infection of the eye five months previous which was treated successfully with a stock vaccine.

Physical examination revealed a pale, undernourished thin boy, weighing 108.5 pounds. Examination of the heart and lungs was negative. His nasopharynx showed no abnormality. His abdomen was markedly tender, especially in the left lower quadrant. There was no rigidity, and no masses were palpable.

Proctosigmoidoscopy performed on January 4, 1938, gave the following findings: The entire rectosigmoid mucosa showed marked redness with some false membrane formation. In places it was granular and there were numerous ulcerated areas, varying in size from a pin point to 1 cm. in diameter.

Cultures taken directly from the ulcerations showed the presence of many colonies of *B. coli* and *Staphylococcus albus*, but no streptococci. Cultures taken from the nasopharynx showed the presence of colonies of *Streptococcus viridans* and *Staphylococcus albus*. A vaccine was made of the *Staphylococcus albus*, this being the only organism present both in rectosigmoid and throat cultures.

Upon admission to the hospital the patient was given vaccine as well as other supportive therapy, such as liver extract injections, medicated enemas, and one transfusion. During the five weeks the patient remained at the hospital he failed to improve, the number of his bloody stools increased, and he had a further loss of weight, his lowest weight being 84 pounds. Following his dismissal he returned home where the vaccine therapy was continued. He also received several small transfusions at home. He began to improve about March 1, 1938, after having received 20 vaccine injections. Twelve more injections were given after this time. When seen on April 20, 1938, his weight was 104 pounds (a gain of 20 pounds in two months), and his general condition was very good. Bowel evacuations were normal.

Physical examination at this time was negative. The patient had no abdominal pain or discomfort: His hemoglobin was 68 per cent.

Proctosigmoidoscopy showed normal mucosa throughout. Cultures from the rectosigmoid mucosa showed the presence of *B. coli*, but no growth of *Staphylococcus albus*. Nose and throat cultures showed many colonies of *Streptococcus viridans* and a small number of *Staphylococcus albus*.

On September 8, 1938, his weight was 120 pounds; his hemoglobin was 78 per cent, his condition was excellent, and his bowel movements were normal.

The patient presented himself again on December 3, 1939. His health was still good. His weight was 137.5 pounds and his hemoglobin was 86 per cent. He claimed that this time he had one normally formed bowel movement daily.*

*Since presenting this paper the patient was seen several times thereafter.

COMMENT

What relation these various organisms from the nose, throat, and rectosigmoid, as indicated in our studies, bear to the etiology of nonspecific ulcerative colitis we are not prepared to state. Whether by giving these various vaccines we are preventing upper respiratory infections, and in that way preventing exacerbations, we do not know. However, this seems to be the possible explanation of the rationale of our treatment. Nevertheless, we will continue to use this form of treatment, and hope that others will utilize it until such time as the specific etiology of nonspecific ulcerative colitis will be definitely established. We are of the opinion, as Paulson believes, that in those particular cases of ulcerative colitis, where amoeba, *B. dysenteriae*, and diplostreptococci of Bargen are found, the resulting ulcerative colitis is secondary to these organisms.

CONCLUSIONS

We have discussed a series of cases of nonspecific ulcerative colitis presenting in detail one case. These cases were successfully treated with vaccines prepared from cultures from the nose, throat, and rectosigmoid containing only those organisms that were found simultaneously in the nasopharynx and rectosigmoid. We have had no recurrence in following these cases from a period of nineteen months to four years.

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CLINICAL CHEMISTRY

THE EFFECTS OF INSULIN, METRAZOL, AND ELECTRIC SHOCK ON BLOOD PYRUVATE, LACTATE, AND GLUCOSE*

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IT HAS been observed by Rivers[1] that intravenous administration of large doses of vitamin B_1 early in stupor is often effective in preventing early convulsions with patients undergoing the insulin shock treatment for schizophrenia. Demole[1a] had noted that administration of vitamin B_1 to rabbits during twenty-four hours previous to insulin injection usually prevented convulsions. These results suggested a possible correlation between the effects of insulin and other convulsive agents and the metabolism of carbohydrate via pyruvic acid, since it is well established that vitamin B_1, or rather the vitamin pyrophosphate, is concerned in pyruvic acid breakdown. A study has, therefore, been made of the changes in blood pyruvate, lactate, and glucose, which occur during insulin shock, after small insulin injections in normal subjects, and after metrazol and electric shock convulsions.

METHODS

Insulin was administered intramuscularly; metrazol and curare were administered intravenously. The electric shock treatment consisted in the brief application of alternating current at about 120 volts through electrodes applied to the sides of the forehead by a slight modification of the procedure of Cerletti and Bini.[2] Vitamin B_1 was injected intravenously.†

For each set of determinations about 10 c.c. of blood were drawn from the antecubital vein, the tourniquet being removed, directly into a test tube containing 40 mg. of potassium oxalate and 0.2 c.c. of 25 per cent sodium iodoacetate using a suction apparatus. Quick mixing with iodoacetate prevented the disappearance of pyruvate which otherwise occurs very rapidly.[3] The presence of iodoacetate also prevented glycolytic increases of lactic acid. As soon as possible 2 c.c. of the sample were deproteinized with tungstic acid by the Haden modification of the Folin-Wu method, and 5 c.c. were deproteinized by running into 20 c.c. of 10 per cent trichloracetic acid. All determinations were made in duplicate.

Glucose was determined on the tungstic filtrates by the method of Benedict, using the Klett-Summerson photoelectric colorimeter. With this colorimeter it was found that the rate of color development and the time for the color to

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†Vitamin B_1, thiamin chloride, was kindly supplied to T.D.R. by Eli Lilly and Company.

reach its maximum value depended upon the amount of glucose in the sample. In the usual method the solutions are cooled before adding the color reagent. Under these conditions, except with very small amounts of glucose, the color was still deepening rapidly ten minutes after adding the color reagent. Maximum color was generally reached, and readings could best be taken after fifteen to twenty-five minutes; after thirty minutes the color faded slowly.

In the presence of 0.05 per cent iodoacetate the color was found to develop more slowly; it never reached the correct value. However, if, instead of cooling the reaction mixture after the six-minute heating, the color reagent was added to the hot solution, the color developed rapidly. Under these conditions readings taken twenty minutes after adding the color reagent and diluting, gave correct values when compared with two standard solutions containing iodoacetate and run in the same manner.

Lactic acid was determined on the trichloracetic acid filtrates, after removal of sugar with copper sulfate-copper hydroxide, by the method of Barker and Summerson.[4] The presence of iodoacetate in the solutions to be analyzed had no effect on the results.

Pyruvic acid was determined on trichloracetic acid filtrates by the method of Lu,[5] as modified by Bueding and Wortis,[3] the directions of the latter authors being followed exactly. Tungstic acid itself did not interfere with the determination of pure pyruvate solutions. But tungstic acid filtrates from blood could not be used for the pyruvate determinations, since low values were always obtained and, when known amounts of pyruvate were added to blood before deproteinizing with tungstic acid, the recovery was low. Evidently the protein precipitated with tungstic acid carries down some pyruvate. Complete recovery was obtained after trichloracetic acid precipitation. The trichloracetic filtrates could be kept for several days before analysis without appreciable change. Oxalate and iodoacetate did not interfere with the determination. While the method is more specific than the Clift and Cook[6] determination of bisulfite-binding substances and gives much lower values with blood,[7] it is not completely specific. It has been assumed in this paper that pyruvate itself is largely responsible for the values obtained, but it must be remembered that α-ketoglutarate and oxaloacetate, and possibly other substances, are included in the values.

RESULTS

The results of all experiments are presented diagrammatically in Figs. 1 to 17. The ordinates for lactic acid and pyruvic acid are respectively twice and twenty times the size of those for glucose.

The legends for the figures describe the material for the various experiments. All persons were initially fasting, except those represented in Figs. 16 and 17. As far as possible persons were initially resting, but the condition of the patients did not always allow this condition to be well controlled.

Lactate.—No consistent changes in the amount of lactate in the blood during insulin stupor were observed, but some fluctuations occurred which could probably be attributed to variations in muscular activity of the patients. In three of four cases of resting normal persons receiving small doses of insulin

(see Figs. 5, 7, 8), slight rises in blood lactate occurred, possibly as a result of extra visceral muscular activity due to the hypoglycemia. Rises of blood lactate in experimental animals treated with insulin have been observed and attributed to increased muscular activity by earlier workers.[8]

Metrazol and electric shock convulsions caused extremely large rises in blood lactate,[9-11] obviously as a result of the intense muscular contractions. The rapidity of the rise after electric shock convulsions is noteworthy. Possibly the same occurs during metrazol convulsions, but blood could not be drawn as immediately after metrazol as after electric shock. When the muscular activity was moderated by previous administration of curare, the rise in blood lactate was less marked.

Pyruvate.—Von Euler and Högberg[12] have stated that an hour after administration of 10 units of insulin to normal persons, decreases of blood pyruvate of 75 to 100 per cent were found. Though we used a similar method for pyruvate determination, we never found any appreciable decrease in blood pyruvate as a result of administration of small or large doses of insulin.

Fluctuations in blood pyruvate followed closely the direction of the fluctuations in blood lactate, and it seems clear that the amounts of these two substances are related to each other and are affected similarly by muscular activity and not specifically by insulin or the other agents. The fluctuations in pyruvate, however, were always much less wide than those of lactate. Johnson and Edwards[13] found increases of lactate and pyruvate in blood and urine with young men after severe exercise and suggested that the pyruvate was produced, in association with lactate, by the active muscles; this suggestion was shown to be true by Bollman and Flock[14] in experiments with exercising rat muscle.

It is reasonable to believe that, with large doses of insulin, consciousness is lost and other brain functions are affected as a result of a lack of oxidizable substrate (glucose) for the oxidations necessary to maintain brain activity.[15] However, no correlation was observed between the state of consciousness and the level of blood pyruvate or lactate during insulin or the other treatments. Wortis and Goldfarb[16] observed that injections of lactate into patients in insulin coma had little effect on the oxygen consumption of the brain and did not arouse the patient. Mann and Magath[17] were unable to relieve the convulsions of hepatectomized animals with sodium lactate. With hepatectomized abdominal eviscerated rabbits, Maddock and co-workers[18] found that the cortical electrical potentials could be restored by intravenous injections of glucose but not of a number of other substances including pyruvate. With patients in insulin hypoglycemia Himwich and associates[19] found no significant absorption of lactic acid from the blood circulating through the brain, and Wortis and Goldfarb[20] found no sign of metabolism of injected lactate or pyruvate by the brain. It thus seems that lactate and pyruvate in the blood are not available for oxidations in the brain, although the brain is well known to oxidize these substances in vitro.

Injections of large doses of vitamin B_1 into patients in insulin stupor produced no definite effect on blood pyruvate or glucose* within the period of

*Demole[18] found that the previous administration of the vitamin to rabbits often diminished the hypoglycemia caused by insulin.

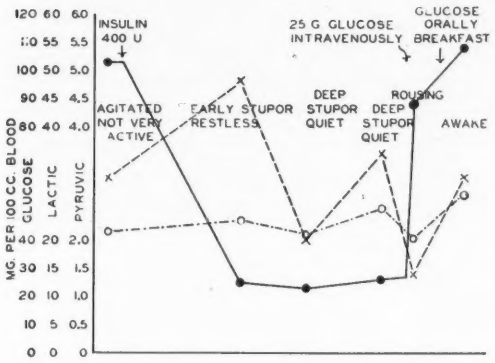


FIG. 1

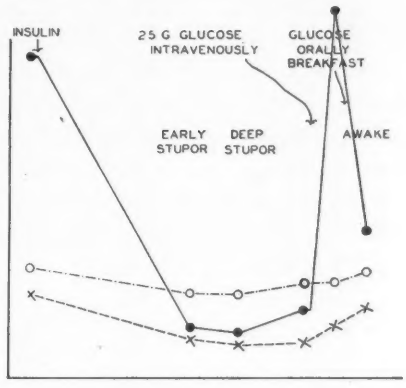


FIG. 2

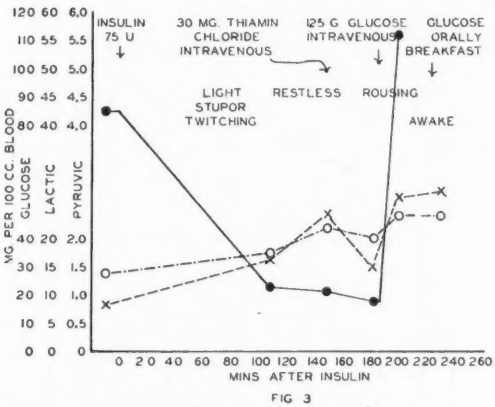


FIG. 3

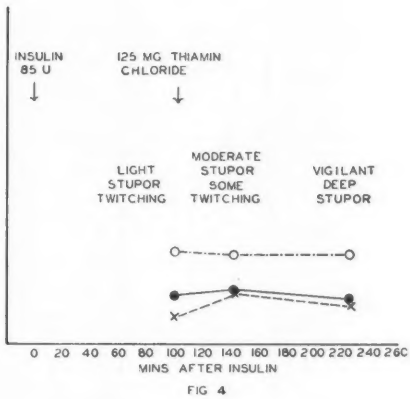


FIG. 4

Insulin shock treatments.

Glucose •——•, Lactate x----x, Pyruvate o-.-.-o.

Fig. 1.—L. K., male, 20 years old, paranoid schizophrenic.
Fig. 2.—N. N., male, 34 years old, paranoid schizophrenic.
Fig. 3.—G. W., female, 22 years old, paranoid schizophrenic.
Fig. 4.—J. I., male, 15 years old, paranoid schizophrenic.

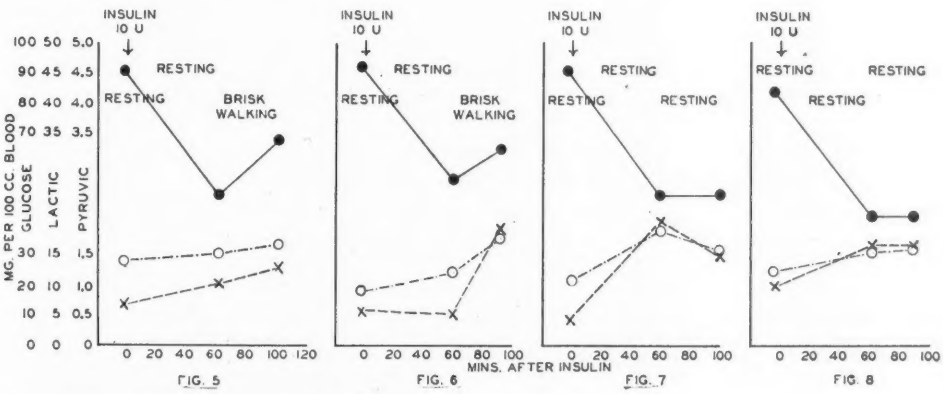


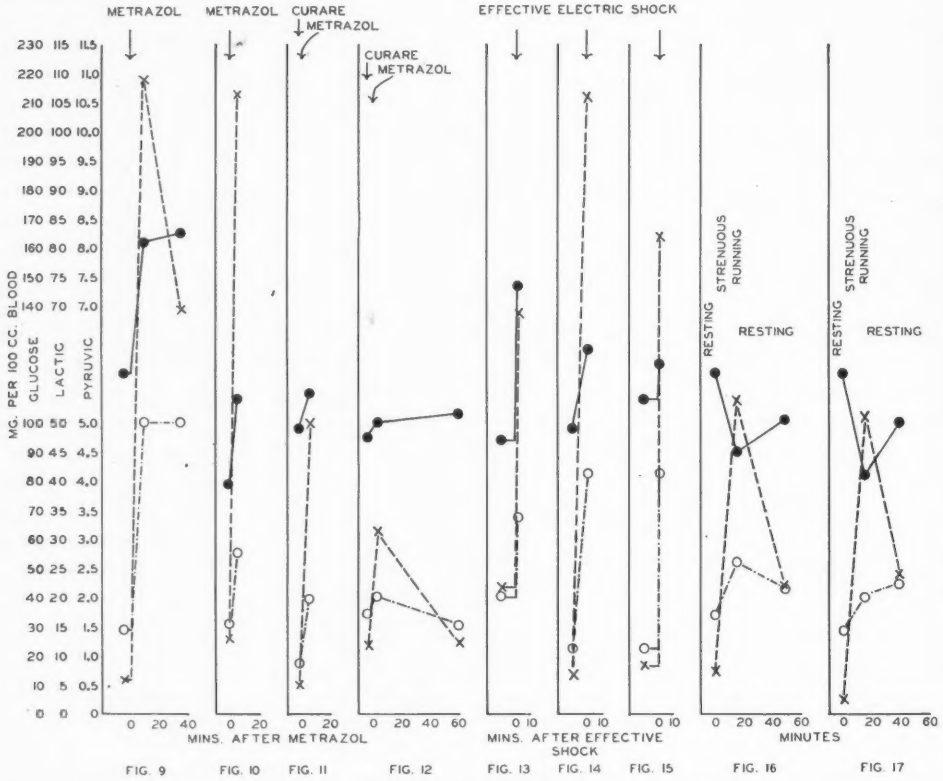
FIG. 5 FIG. 6 FIG. 7 FIG. 8

Insulin in small doses to normal subjects.

Glucose •——•, Lactate x----x, Pyruvate o-.-.-o.

Fig. 5.—A. E., male, aged 37 years.
Fig. 6.—B. L., male, aged 24 years.
Fig. 7.—F. E., female, aged 34 years.
Fig. 8.—B. P., female, aged 20 years.

the experiment. Platt and Lu[7] found a lag period of several hours before the high blood pyruvate of vitamin B_1-deficient patients was affected by curative small doses (5 mg.) of the vitamin. The observation of Rivers[1] on the effect of vitamin B_1 on insulin convulsions remains unexplained by the present work. A possible explanation is that during insulin hypoglycemia slight available amounts of glucose continue to be metabolized by the brain, producing within the cells suboptimal amounts of intermediary metabolites, the oxidation of which can be sufficiently stimulated by excess vitamin B_1 to produce more normal conditions.



Metrazol convulsions with (Figs. 11 and 12) and without (Figs. 9 and 10) partial curarization.

Glucose •——•, Lactate x----x, Pyruvate o-.-.-o.

Fig. 9.—G. L., female, aged 53 years, involutional melancholia.
Fig. 10.—M. R., female, aged 40 years, involutional melancholia.
Fig. 11.—V. H., male, aged 50 years, involutional melancholia.
Fig. 12.—R. H., male, aged 54 years, involutional melancholia.

Electric shock.

Glucose •——•, Lactate x----x, Pyruvate o-.-.-o.

Fig. 13.—F. K., female, 48 years old, involutional melancholia.
Fig. 14.—Z. S., female, 64 years old, manic-depressive depressed.
Fig. 15.—R. M., female, 52 years old, involutional melancholia.

Brief exercise to exhaustion on normal subjects.

Glucose •——•, Lactate x----x, Pyruvate o-.-.-o.

Fig. 16.—B. P., female, 20 years old. About two hours after lunch.
Fig. 17.—F. E., female, 34 years old. About two hours after lunch.

Bueding and Wortis[3] found that the pyruvic acid content of normal blood fell in the range 0.77 to 1.16 mg. per 100 c.c., averaging 0.98. It will be noticed that our initial resting figures are mostly appreciably higher, 0.9 to 2.2, averaging 1.4. The difference may be due to the fact that Bueding and Wortis' subjects were probably more thoroughly rested than ours.

Glucose.—Short severe muscular effort is well known to raise the fasting blood sugar.[21] Blood glucose rose rapidly and considerably in metrazol (see Meduna and Rohny;[11] Loman and co-workers;[22] and Redlich[23]) and electric shock convulsions. Exercise after small doses of insulin to normal persons also raised the blood sugar considerably (Figs. 5 and 6). During brief exhausting exercise of nonfasting normal persons, the blood sugar fell steeply and recovered during rest (Figs. 16 and 17).

SUMMARY

Neither insulin in small or large doses, nor metrazol or electric shock treatments, have any specific effect on the level of blood pyruvate, and probably none on the lactate level, in human beings. The concentration of these substances bore no relation to the blood sugar level nor to the condition of brain functions.

Blood pyruvate and lactate rose and fell together, and the rises seemed to be the result only of muscular activity. In metrazol and electric shock the rises of both substances, and of blood glucose, were very high; curare decreased the effect of metrazol convulsions on blood lactate, pyruvate, and glucose. The changes in blood pyruvate were less marked than those of lactate.

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BLOOD SPECIFIC GRAVITY STUDIES*

Relationship of Specific Gravity of Whole Blood to Specific Gravity of Plasma, Red Blood Cell Count, Hematocrit, and Hemoglobin as Indicators of Hemoconcentration*

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THERE has been an increasing familiarity with specific gravity studies, red blood cell counts, hemoglobin determinations, and hematocrit studies as methods of measuring hemoconcentration and hemodilution in shock and related conditions. Many investigators feel that specific gravity study is the simplest and most practical method of recognizing and following hemoconcentration during the course and treatment of shock. The method of Barbour and Hamilton[1] has placed this means of study on a practical accurate basis, and Scudder[2] has popularized the method in the study of shock. In a previous publication,[3] the specific gravity method has been demonstrated to be not only an excellent method of studying the course of hemoconcentration during experi-

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mental shock, but also a means of recognizing relative blood volume changes. This latter application depends upon the assumption of a relatively constant erythrocyte specific gravity. In a series of experiments on dogs in the study of shock, it was found that the erythrocyte specific gravity varied only slightly in normal animals and during the course of shock.

It has been felt necessary to investigate the erythrocyte specific gravity in human beings and to determine whether this factor is as nearly constant as it is in dogs. In this study also particular attention has been paid to the linear relationship between the various methods of indicating hemoconcentration, as well as the relative efficacy of each of these methods.

METHODS

A series of 20 normal persons with red blood cell count over 4,000,000 and a series of 10 patients, most of whom had some form of anemia, were studied to determine the specific gravity of the red blood cells. Blood was obtained by venepuncture with minimal stasis. Heparin was used as the anticoagulant. On each sample, red blood cell counts, hemoglobin by Sanford and Sheard method[4] using a photoelectric colorimeter, hematocrit using Wintrobe tubes,[5] and specific gravity of whole blood and plasma using the method of Barbour and Hamilton[1] were carried out. Mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were calculated from the red blood cell count, hemoglobin, and hematocrit. The specific gravity of the red blood cells was calculated as follows:[3]

$$\text{Sp. gr. of R. B. C.} = \frac{(\text{Sp. gr. whole blood} \times 100) - [\text{Sp. gr. plasma} \times (100 - \text{Hematocrit})]}{\text{Hematocrit}}$$

The efficacy of protein concentration as an indicator of hemoconcentration was studied in a series of dogs in which shock was experimentally produced during which hemoconcentration studies were carried out.

RESULTS

Table I shows the results of the studies on human beings. The specific gravity of red blood cells shows only minor variation in normal human beings, varying from 1.0936 to 1.0998, with an average of 1.0971. In patients with anemia, however, the variation was somewhat greater, from 1.0868 to 1.1045, this variation bearing slight but not absolute relationship to the mean cell hemoglobin concentration. It would appear that a close relationship between mean cell hemoglobin concentration and specific gravity of red blood cells exists, and the absence of such may be due to technical variations. It would seem that, although in anemia and other abnormal conditions, the red blood cell specific gravity may vary from the average, in the same person the factor is sufficiently constant that the specific gravity method is an accurate means of indicating relative blood volume changes. A study of the literature shows that Oestreich[6] in 1931 studied the specific gravity of blood using methods susceptible to serious criticism; he found the specific gravity of erythrocytes to be around 1.09, a somewhat lower figure than that obtained here.

TABLE I

BLOOD STUDIES ON NORMAL PERSONS, ANEMIC PATIENTS, PATIENTS WITH MILD IRON DEFICIENCY ANEMIA, AND ONE WITH POLYCYTHEMIA VERA

	R.B.C. COUNT IN MILLIONS	HEMOGLOBIN GM./100 C.C.	HEMATOCRIT	MCV	MCH	MCHC	SP. GR. PLASMA	SP. GR. BLOOD	SP. GR. ERYTHROCYTES
Normal	5.07	15.85	44.0	86.8	31.3	36.0	1.0264	1.0560	1.0936
Normal	4.76	14.15	47.0	98.7	29.7	30.1	1.0252	1.0582	1.0953
Normal	4.85	15.85	45.5	93.8	32.7	35.6	1.0264	1.0571	1.0954
Normal	4.60	15.85	43.0	93.5	34.4	36.9	1.0257	1.0557	1.0956
Normal	5.38	16.10	47.5	88.3	29.9	33.9	1.0251	1.0589	1.0962
Normal	4.97	16.65	46.8	94.2	33.5	35.6	1.0257	1.0588	1.0964
Normal	5.50	16.90	47.5	86.4	30.7	35.6	1.0249	1.0589	1.0965
Normal	4.64	14.70	43.0	92.7	31.7	34.2	1.0278	1.0573	1.0965
Normal	4.22	14.15	39.5	93.6	33.5	35.8	1.0253	1.0534	1.0965
Normal	4.30	14.60	39.5	91.9	34.0	37.0	1.0251	1.0533	1.0965
Normal	4.84	14.70	42.0	86.7	30.4	35.0	1.0260	1.0559	1.0971
Normal	4.47	14.45	39.0	89.2	32.3	37.1	1.0266	1.0542	1.0974
Normal	5.53	15.85	46.0	83.2	28.7	34.5	1.0266	1.0594	1.0979
Normal	4.06	14.15	38.0	93.6	34.9	37.3	1.0272	1.0541	1.0979
Normal	5.09	16.10	45.0	88.4	31.6	35.6	1.0260	1.0584	1.0980
Normal	5.13	15.55	43.5	84.8	30.3	35.1	1.0250	1.0569	1.0984
Normal	5.08	15.55	47.5	83.5	30.6	32.7	1.0293	1.0622	1.0986
Normal	4.86	14.15	39.0	80.2	29.1	36.3	1.0240	1.0533	1.0992
Normal	5.77	17.40	48.0	83.2	30.2	36.3	1.0276	1.0622	1.0998
Normal	5.09	15.55	47.0	92.3	30.6	33.1	1.0258	1.0603	1.0993
Patient	3.70	9.15	31.0	83.8	24.7	29.5	1.0251	1.0442	1.0868
Patient	2.53	8.20	29.0	114.6	32.4	28.3	1.0246	1.0433	1.0891
Patient	8.52	25.80	78.0	91.5	30.3	33.1	1.0269	1.0758	1.0896
Patient	4.20	13.25	36.5	96.1	34.9	36.5	1.0251	1.0521	1.0911
Patient	4.85.	13.85	45.5	93.8	28.5	30.5	1.0279	1.0569	1.0916
Patient	4.43	12.90	38.5	86.9	29.0	33.5	1.0247	1.0521	1.0958
Patient	4.42	12.60	37.1	83.9	28.5	33.9	1.0276	1.0535	1.0973
Patient	4.62	13.50	38.5	83.5	29.3	35.1	1.0259	1.0542	1.0995
Patient	4.70	15.3	41.7	88.7	32.6	36.7	1.0263	1.0580	1.1024
Patient	3.36	11.7	32.9	97.9	34.8	35.6	1.0263	1.0470	1.1045

With the average specific gravity of human erythocytes known, the hematocrit may be calculated from the specific gravity of whole blood and plasma as follows:

$$\text{Hematocrit} = \frac{(\text{Sp. gr. whole blood} \times 100) - [\text{Sp. gr.} \times (100 - \text{Hematocrit})]}{1.0971}$$

The relationship between specific gravity of whole blood and the hematocrit, red blood cell count, and hemoglobin would be expected to be fairly constant. Since, however, the specific gravity of whole blood is partly dependent upon the specific gravity of the plasma, there should be some departure from this relationship when this latter factor is not taken into account. For practical purposes, in spite of this, the hematocrit, red blood cell count, and hemoglobin bear a distinct mathematical relationship to, and can be fairly accurately estimated from, the specific gravity of the whole blood. The relationship between these is shown in Figs. 1, 2, 3. This relationship between specific gravity of whole blood and the hematocrit, hemoglobin, and red cell count may be expressed as follows:

(1) Hematocrit = (Sp. gr. whole blood – 1.0457) × 1,000 + 32.

(2) Hemoglobin = (Sp. gr. whole blood – 1.0470) × 386 + 11.

(3) Red blood cell count in millions = (Sp. gr. whole blood – 1.0466) × 14.5 + 3.5.

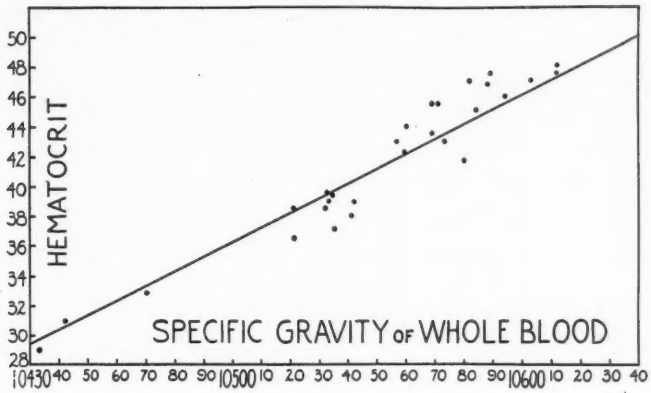

Fig. 1.

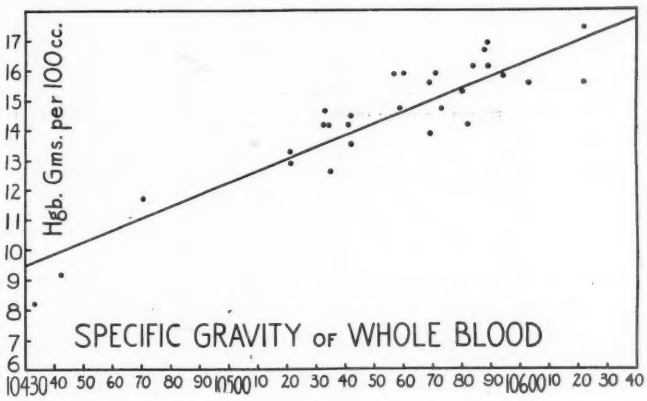

Fig. 2.

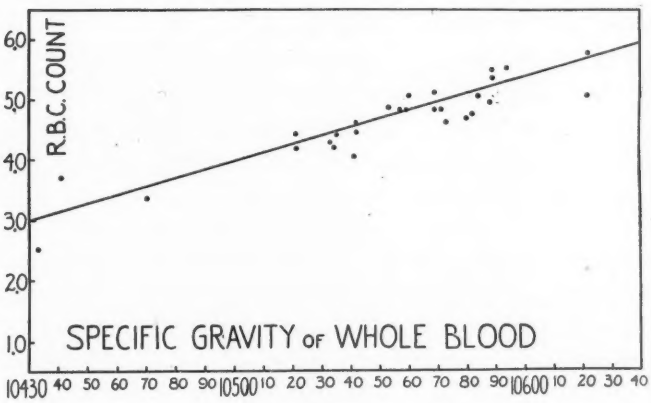

Fig. 3.

Naturally these equations will yield results slightly in error of the actual values, but these variations do not exceed the limits of technical error by any appreciable extent. Because of this rather accurate correlation between the specific gravity of whole blood and hematocrit, hemoglobin, and red blood cell count, it would seem that they are more or less uniformly efficacious as indicators of hemoconcentration. The value of these studies of peripheral blood as measures of hemoconcentration and relative blood volume change has been further accentuated by recent studies of Ebert and Stead[7] which showed the relative constancy of circulating red blood cell volume in human beings under various circumstances.

TABLE II

HEMOCONCENTRATION STUDIES IN DOGS IN WHICH SHOCK WAS INDUCED BY INTRAPERITONEAL INJECTION OF HYPERTONIC SALINE (25%)

DOG		R.B.C. COUNT IN MILLIONS	HEMOGLOBIN GM./100 C.C.	HEMATOCRIT	PROTEIN GM./100 C.C.	SP. GR. BLOOD	PROTEIN	HEMATOCRIT	HEMOGLOBIN	R.B.C. COUNT
							PERCENTAGE INCREASE			
1	Control	4.2	10.15	33	5.37	1.0465				
	Shock	9.64	20.15	60.5	6.09	1.0691	13.4	83.3	98	127
2	Control	4.76	12.45	32	5.58	1.0529				
	Shock	10.74	24.0	61.5	6.53	1.0817	17	92	92.8	125
3	Control	5.16	12.95	38.5	6.12	1.0509				
	Shock	8.43	18.9	56.5	5.85	1.0649	–5	46.7	46.2	63.3
4	Control	5.03	12.35	36.0	6.56	1.0500				
	Shock	7.95	17.9	51.5	6.7	1.0642	3.6	43	45	58
5	Control	6.37	17.15	48.5	6.39	1.0610				
	Shock	9.63	23.15	70	7.28	1.0749	14.1	44.3	35	51

Table II represents hemoconcentration studies in five dogs in which shock was induced by intraperitoneal injection of hypertonic saline. The percentage increase of hemoglobin, hematocrit, and red blood cell count from control studies to shock values demonstrates the relative efficiency of each method of determining hemoconcentration. It will be seen that fairly accurate correlation in the values was obtained. Protein concentration was estimated from the specific gravity of the plasma according to the method of Weech, Reeves, and Goettsch.[8] A comparison of the percentage change of protein concentration from control to shock values with corresponding values of other measures of hemoconcentration shows the lack of efficiency of protein concentration as a measure of hemoconcentration or relative blood volume change. This is to be expected, since proteins are lost from the circulating blood during the development of shock.

SUMMARY

1. The average specific gravity of normal human erythrocytes is 1.0971. This factor is fairly constant in different persons, but in anemia there frequently is a greater departure from the average than in normals.

2. The hematocrit may be calculated from the specific gravity of whole blood and of plasma in human beings. Also a fairly accurate estimate of red blood cell count, hemoglobin, and hematocrit may be made from the specific gravity of whole blood alone, since these values bear a constant relationship to one another.

3. Specific gravity of whole blood, hemoglobin concentration, hematocrit, and red blood cell count are uniformly efficacious as indicators of hemoconcentration and relative blood volume change.

4. Protein concentration or specific gravity of plasma is not a satisfactory indicator of hemoconcentration during the course of shock, since protein is lost from the circulating blood.

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INDOLURIA IN RHEUMATOID ARTHRITIS*

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INDOLE is formed by bacterial action upon protein. This action may take place in the gastrointestinal tract, the urinary tract, or in some focus of infection.[3] Normally, indole is oxidized in the body to indoxyl, which is conjugated with potassium hydrogen sulfate, with the formation of indican that is excreted in the urine. The indoxyl detoxifying mechanism is located in the liver.

In *normal* persons under *normal* conditions, free indole does not appear in the urine. In over 100 determinations on normal persons, urinary indole was found in only one, and then only upon one occasion.[4] On the other hand, indoluria was encountered in various pathologic conditions.[2, 4]

Vaughan[11] was unable to detect the presence of indole in specimens of urine from diseased persons. However, Vaughan,[12] and Carnes and Lewis,[3] point to the possibility that the urine may contain a substance that might be converted into indole by the action of *E. coli*. These investigators observed that indole-negative urines may become positive upon standing.

Deficient oxidation, impaired liver function, sulfur deficiency, excessive indole production in the intestinal tract, and many other concepts were advanced as theoretical possible causes for the appearance of free indole in the urine.[3, 4]

*This study was conducted from February 2 to July 15, 1938.

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Forbes and Neale[4] reported indoluria in a variety of pathologic conditions, including rheumatic fever, gonococcal, infectious, rheumatoid, and hypertrophic arthritis. These investigators used steam distillation in the presence of tartaric acid, followed by colorimetry, to determine quantitatively the indole in the urine. Values less than 0.05 mg. per liter of urine were regarded as a trace. Most cases ran from 0.5 to 1.5 mg. per liter of urine.

As indoluria might be evidence of the liver failing in its detoxifying action, possibly because of sulfur deficiency, it seemed logical to Forbes and Neale and their collaborators[5] to give patients with chronic arthritis a diet that contained a high proportion of sulfur amino acids. It was noted that clinical improvement was accompanied by a decrease and eventual disappearance of the indoluria.

In contrast, it seemed improbable to Basinski and Lewis[2] that indoluria should be related to a lack of sulfur needed for conjugation. In their patients having indoluria, the cystine level in the fingernails was found to be normal.

The demonstration of indoluria in patients with rheumatoid arthritis by Forbes and Neale,[4] prompted me to test the urines of 88 patients with rheumatoid arthritis for the presence of free indole. The patients, consisting of 46 females and 42 males, were partly from my private practice, partly from the People's Hospital at Pistany Spa, Czechoslovakia. This hospital, equipped with 600 beds, is dedicated to the treatment of panel patients suffering from chronic arthritis and other rheumatoid conditions. The patients stayed three to four weeks at the hospital, the average duration of the "spa cure." The spa treatment consists of the use of naturally hot sulfur springs and of the sulfur-containing mud of volcanic origin, the latter being administered in the form of mud baths and packs. The rationale and technique of the spa treatment were described in a previous article.[10]

Considering that sulfur is absorbed through the skin,[1, 7, 8] and that its absorption had been demonstrated from the mud applied to the shaved skin of a rabbit,[6] I attempted to investigate not only the presence of free indole in the urines of patients with rheumatoid arthritis, but also its fate in the urine of patients who take a "spa cure." However, I was unable to complete the latter part of my study.

The samples of urine, obtained from the patients at any hour throughout the day, and at any period in the course of the spa treatment, were collected as follows: After the glans penis and the urethral orifice of the male patient had been washed, the urine was collected in two portions. The first specimen of urine was discarded, the second one was collected in a sterile container and promptly tested for indole. The female patients cleansed their external genitals, and voided into two containers, the second of which was sterile and its contents were used for indole determination, which analysis was made within thirty to sixty minutes. The method of analysis used by Forbes and Neale[4] was employed in the study on indoluria.

The analytical results of the examined urine specimens obtained from 88 patients with rheumatoid arthritis were as follows: In the 46 females, the values of indole were between 0.3 and 1.8 mg. per liter of urine; in the 42

males, the indole values were between 0.2 and 1.94 mg. per liter of urine. Less than 1 mg. of indole per liter of urine was found in 32 females (69.6 per cent), and in 31 males (73.8 per cent). More than 1 mg. of indole per liter of urine was found in 14 females (30.4 per cent) and in 11 males (26.2 per cent).

The results reported here are in agreement with those of Neale[9] who, recently, in a series of 50 cases of proliferative arthritis in female patients obtained urine aseptically by catheterization and demonstrated free indole in urine which was shown to be sterile by culture. This indoluria disappeared when the patients responded to treatment.

SUMMARY

Free indole was shown to be present in the freshly voided urine samples obtained from 88 patients with rheumatoid arthritis. These results confirm the observations reported by Forbes and Neale.[4]

I wish to acknowledge the cooperation of Dr. Anna Igumova, Head of the Chemical Research Laboratory at Pistany Spa. I gratefully offer an expression of appreciation to her.

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135 WEST 79TH STREET

THE IODINE CONTENT OF BLOOD, URINE, AND SALIVA OF NORMAL PERSONS IN THE NEW YORK CITY AREA*

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SINCE the numerous studies on the iodine content of body fluids have been adequately reviewed in the recent books by Elmer,[1] McClendon[2] and Salter,[3] only the pertinent literature will be referred to in this report.

MATERIAL AND METHODS

Blood, urine, and saliva were obtained from 22 normal persons of whom 9 were males and 13 were females. Their ages ranged from 13 to 51 years. The usual hospital personnel (physicians, nurses, technicians, etc.) served as a major source of material but those assigned to surgery were uniformly excluded because of probable contact with iodine. No attempt was made to standardize the antecedent diet. All materials used for the collection of specimens, such as syringes, needles, and containers, after thorough cleansing, were rinsed with iodine-free distilled water. Iodine-free alcohol was used to sterilize the skin prior to venipuncture.

Urine collections were made over a twenty-four-hour period; blood and saliva were obtained usually after breakfast, and approximately twelve hours after the collection of the urine was begun. The method of Trevorrow and Fashena[4] was used throughout.

RESULTS

The results are given in Table I. The total blood iodine ranged from 3.1 to 8.4 micrograms per 100 ml. of whole blood, with a mean of 5.9 ± 1.3. The concentration of iodine in the urine varied from 6.2 to 56.7 micrograms per 100 ml., with a mean of 21.1 ± 16.7. The total output of iodine in the urine for twenty-four hours ranged from 18.0 to 483.1 micrograms, with a mean of 190.8 ± 138.0. The salivary iodine varied from 3.5 to 24.0 micrograms per 100 ml., with a mean of 10.2 ± 5.1.

DISCUSSION

It is generally appreciated that the normal range for blood iodine reported by some of the earlier investigators is undoubtedly too high. Riggs and Man[5] have recently analyzed critically the various procedures and have pointed out the discrepancies inherent in the older methods of chemical analyses. The comparatively high normal values recorded in the literature may further be ascribed to the selection of persons who inadvertently ingested iodine or who were ex-

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posed to it in some form. Greene and one of us (M. B.)[6] have stressed this point. In the present series, the average concentration of 5.9 ± 1.3 micrograms of iodine per 100 ml. of whole blood agrees closely with the findings of some recent investigators[7] and is slightly higher than that recorded by another group.[8]

Baumann and Metzger[8c] maintained that the blood iodine of males was higher than that of females, an observation which was not confirmed by Fashena,[7b] Turner and co-workers,[7c] and Riggs and Man.[5] The present studies are in agreement with the latter group. The average blood iodine in the 9 males was 5.7 micrograms per 100 ml. and in the 13 females, 6.0 micrograms per 100 ml.

TABLE I

THE IODINE CONTENT OF BLOOD, URINE, AND SALIVA OF NORMAL PERSONS

SUBJECT NO.	AGE	SEX	BLOOD IODINE (μg/100 ml.)	URINE			SALIVARY IODINE (μg/100 ml.)
				VOLUME (ml./24 hr.)	IODINE (μg/100 ml.)	IODINE (μg/24 hr.)	
1	40	M	4.4	1,880	19.1	359.1	9.3
2	26	M	7.0	1,200	27.7	332.4	9.8
3	28	F	6.0	2,640	10.2	269.3	20.4
4	38	F	6.9	640	54.6	349.4	13.4
5	24	F	6.8	630	6.2	39.1	10.5
6	23	F	8.4	560	7.5	42.0	5.5
7	26	M	3.1	273	6.6	18.0	6.7
8	22	F	6.7	490	11.5	56.4	7.8
9	42	F	5.7	530	21.9	116.1	6.7
10	24	F	4.9	668	19.2	128.3	10.9
11	13	M	5.7	623	7.0	43.7	7.9
12	51	F	7.0	810	12.6	102.1	24.0
13	14	F	5.4	695	11.8	82.0	
14	25	M	6.9	715	55.8	399.0	15.7
15	29	M	6.5	1,695	14.8	250.9	3.8
16	25	F	3.6	1,071	10.3	110.3	3.5
17	25	F	5.6	761	14.7	111.9	6.1
18	32	M	5.5	852	56.7	483.1	6.6
19	17	F	3.8	1,152			11.1
20	36	M	6.5	990	36.1	357.4	15.0
21	28	F	7.5	765	30.6	234.1	14.7
22	30	M	5.4	1,450	8.4	121.8	4.5
Range			3.1-8.4		6.2-56.7	18.0-483.1	3.5-24.0
Mean			5.9		21.1	190.8	10.2
Standard deviation			±1.3		±16.7	±138.0	±5.1

The excretion of iodine in the urine of normal individuals varies directly with the intake of iodine in food and water. The urinary loss of iodine, therefore, is dependent upon the geographic location. In goitrous regions the excretion of iodine in the urine is approximately 50 micrograms or less in twenty-four hours.[9] In the New York City area, where food and water are iodine rich, the present study shows a wide range in the excretion of iodine. The average iodine loss in the urine in the 22 normal persons was 190.8 ± 138.0 micrograms in twenty-four hours.

In contrast to the large number of studies recorded in the literature on blood and urinary iodine, few investigations have been carried out on the concentration of iodine in the saliva.[10] Schneider and Widmann[10b] reported that the salivary iodine may vary from 0 to 362 micrograms per 100 ml., but this range is altogether too wide. In view of the results obtained in the present

studies, some of their subjects were probably ingesting abnormal amounts of iodine. The range observed in the 22 normal persons in this series was 3.5 to 24.0 micrograms per 100 ml. of saliva, with an average of 10.2 ± 5.1.

CONCLUSIONS

1. The concentration of iodine in whole blood of 22 normal persons in the New York City area varied from 3.1 to 8.4 micrograms per 100 ml., with a mean of 5.9 ± 1.3. In 9 males the mean was 5.7 micrograms per 100 ml. and in 13 females, 6.0 micrograms per 100 ml.

2. The concentration of iodine in the urine varied from 6.2 to 56.7 micrograms per 100 ml., with a mean of 21.1 ± 16.7. The total output of iodine in the urine in twenty-four hours varied from 18.0 to 483.1 micrograms, with a mean of 190.8 ± 138.0.

3. The concentration of iodine in the saliva varied from 3.5 to 24.0 micrograms per 100 ml., with a mean of 10.2 ± 5.1.

We are grateful to Samuel Member, B.S. for valuable technical assistance.

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LABORATORY METHODS

GENERAL

MECHANICALLY RECORDING BLOOD SEDIMENTATION CURVES*

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ALTHOUGH credit for discovery of the blood sedimentation test is given to the ancients, the test has been in vogue only since it was accidentally rediscovered in 1918 by the Swedish gynecologist, Robin Fahraeus, in his search for a blood test for pregnancy. Since then no less than forty different techniques have been suggested to perform this very simple test. A standard method would certainly enhance its clinical value.

During recent years efforts have been made by several investigators to record mechanically the blood sedimentation phenomenon. Up to the present three such methods have been devised. These three methods are fundamentally alike in that they are based on the principle that light, which will penetrate plasma, is obstructed by the red blood cell column. The first method devised was the silhouette method. Litten, of Germany,[1] in 1929 exposed a tube of sedimenting blood to a light source. The tube was placed in a slot in front of a kymographic drum, which carried light sensitive paper upon which the increasing column of plasma was recorded as the exposed area of the paper, the lower border of the exposed area revealing the blood sedimentation curve. Sulkowitch[2] devised a very similar instrument in 1934. In 1938 a second method was presented by Lee,[3] who published an article describing the camera method for recording blood sedimentation curves. His device makes use of a lens situated between the blood tube, which is placed in a slot in a light-tight box, and a moving photographic plate which records the image of the illuminated plasma. Nichols[4] reported a similar device with an added improvement of a thermostatic compartment to maintain the blood at normal body temperature. A third method, which as yet has not been published, makes use of a photoelectric cell, exposed to light coming through the blood plasma. The instrument is so designed as to cause the photoelectric cell to move downward with the lower level of blood plasma. This movement in turn operates a pen which records the sedimentation curve as an inked line on paper carried by a kymographic drum.

I have made use of all three of these methods and, in my hands, the simple silhouette method, combined with a thermostatically controlled compartment to maintain the blood at a normal body temperature, proves most satisfactory. The camera method has the disadvantage of making use of an optical system, which

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may make for a source of error not easily detectable. The photoelectric cell method has the distinct advantage of requiring no darkroom technique, inasmuch as the curve is written on paper with an ink tracing, but with this instrument one cannot be sure that the tracing is correct unless one knows that the line of demarcation between plasma and blood cells is definite. With either of the photographic methods, a hazy line of junction will show up as a hazy line, whereas the photoelectric cell method will show up as a sharp line of demarcation regardless of whether or not the zone of junction is clear.

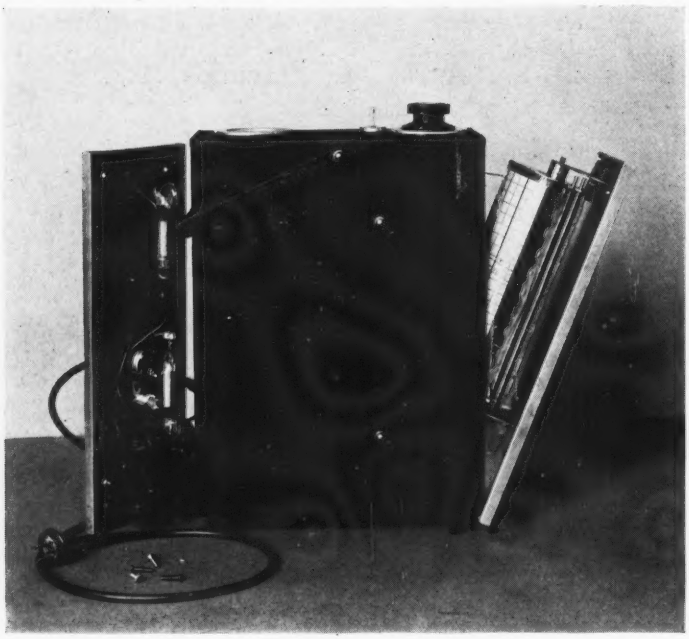

Fig. 1.—Side view of a sedograph with tube in place. The light source compartment has been opened to show the neon tube and transformer. The recording chamber at the right is partly opened, showing the drum.

It has been the common practice of those working with mechanical methods of recording blood sedimentation curves to use the one-hour techniques, especially the Cutler method, probably because it is a well-standardized procedure. I feel that in this point they have been in error. Although the sixty-minute recordings are good methods of ascertaining sedimentation rates when readings are recorded by a laboratory worker, time consumed being a factor, if a machine is to do the test one may just as well see the entire curve without loss of time, and not only a part of the curve as is revealed by the one-hour technique, especially for the bloods of slower sedimentation rates. For instance, two different blood samples which manifest a sedimentation of 5 mm. at the end of one hour in a Cutler tube will also fall 5 mm. in our tube that requires a 30 cm. blood column, because with this slight degree of sedimentation the packing stage influence is not felt. But after the first hour the course of the sedimentation curves of the two bloods may be very different, one sample sedimenting slowly, and the other rapidly. The presedimentation stage is not influenced by the length of the tube. Note in the second sedograph (Fig. 2) that the presedimenta-

tion period requires the entire first hour. Beyond that time the fall may take a slow or a rapid course that can only be determined by reading over a period of time longer than one hour. The longer the tube and time of observation, the more tedious the test but the more accurate the results. Blood samples reveal-

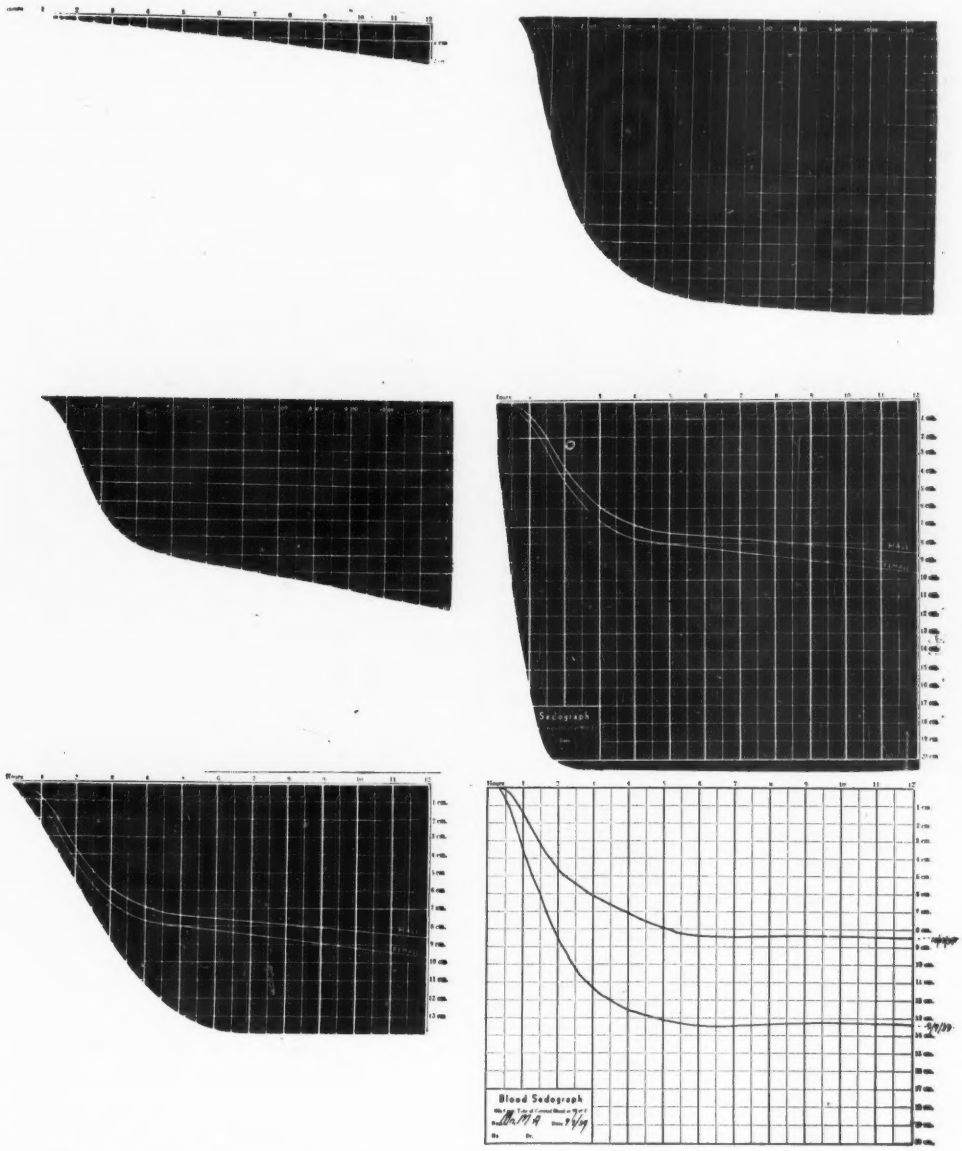

Fig. 2.—Five sedograph records showing different types and degrees of sedimentation curves. In the right lower corner is a piece of tracing paper upon which have been traced two curves from the same individual.

ing identical curves using the one-hour Cutler method have produced unlike sedograph records, one well within the limits of the normal, and the other definitely pathologic.

The silhouette method instrument for recording blood sedimentation at normal body temperature is a device to which I have given the name "sedo-

graph." It is a metal box 5 by 11 by 14 inches, and is divided into three vertical, parallel compartments. In one end compartment is a mercury vapor tube that acts as a light source; in the other end compartment is a slowly revolving drum that carries light sensitive paper which is held in place on the drum by a transparent film mask. On the mask are parallel, horizontal, and vertical black lines, 1 cm. apart, registering as white calibrations the hours of exposure and the centimeters of blood plasma on the final image. The middle compartment contains the blood tube. This compartment is equipped with a heating unit and a thermostatic control which maintains the compartment and the blood tube at

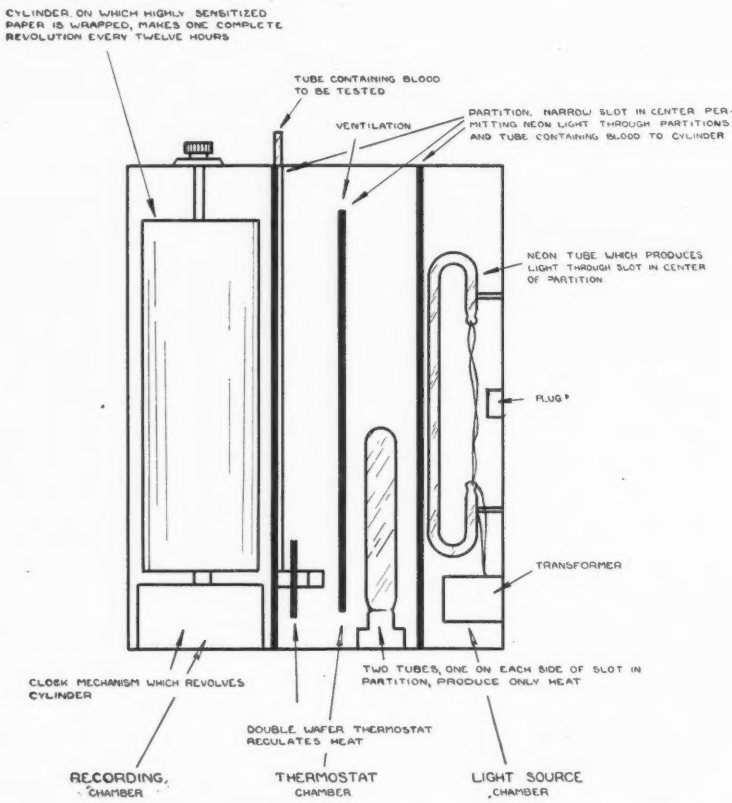
CYLINDER ON WHICH HIGHLY SENSITIZED PAPER IS WRAPPED, MAKES ONE COMPLETE REVOLUTION EVERY TWELVE HOURS
TUBE CONTAINING BLOOD TO BE TESTED
VENTILATION
PARTITION, NARROW SLOT IN CENTER PERMITTING NEON LIGHT THROUGH PARTITIONS AND TUBE CONTAINING BLOOD TO CYLINDER
NEON TUBE WHICH PRODUCES LIGHT THROUGH SLOT IN CENTER OF PARTITION
PLUG
TRANSFORMER
CLOCK MECHANISM WHICH REVOLVES CYLINDER
TWO TUBES, ONE ON EACH SIDE OF SLOT IN PARTITION, PRODUCE ONLY HEAT
DOUBLE WAFER THERMOSTAT REGULATES HEAT
RECORDING CHAMBER
THERMOSTAT CHAMBER
LIGHT SOURCE CHAMBER

Fig. 3.—Lateral diagrammatic drawing of the sedograph instrument, showing the recording chamber on the left, the light source chamber on the right, and the blood-containing thermostatic-controlled chamber between the two.

a temperature of 99.6° F., thus imitating in vivo conditions. Contrary to general opinion, room temperature is a variable which must be taken into consideration if we are desirous of obtaining exact information from blood sedimentation. Room temperature may vary as much as 30° F. in certain parts of the country, which is too great a change to be disregarded if an accurate record is desired. In some bloods I have found sedimentation rates at 100° F. to be as much as doubled what they are at 70° F. To maintain constant temperature, we have found it simpler to use a heating system that will raise the blood above the outer limits of room temperature, rather than to install a cooling system.

The temperature in the thermostatic compartment is controlled by a wafer thermostat and two 25-watt electric light bulbs which cycle on and off, maintaining constant temperature. This type of thermostationary arrangement is used because I have found it to be the one that gives the least lag and the least overshoot at the onset of the test. There is a time interval of about six to ten minutes, depending upon the temperature of the room, during which the thermostationary compartment is warming up to blood temperature. Inasmuch as this is the presedimentation period, I feel that it is unnecessary to warm the compartment by inserting an empty tube into the apparatus just prior to the test.

The records make use of 8½ by 11 inch photographic paper, since this is the size paper most commonly used in hospital charts. This produces an accurate, permanent, actual size image of the sedimentation progress, with more detailed information than do smaller images. This instrument uses ordinary double weight glossy photographic paper, Azo No. 3.

A column of citrated blood 300 mm. in length is used, inasmuch as this is the greatest column of citrated blood that can be accommodated by 8½ by 11 inch paper in those cases with increased sedimentation rates. The length of the tube is a guard against deviation from a strictly upright position, the importance of which has been pointed out by numerous investigators. It is more difficult to be sure that the shorter tubes are perpendicular. We use a tube of 4 mm. inside diameter; tubes of larger diameter transmit the required amount of light with difficulty. This is a convenient sized tube to handle and clean.

The test is run for a period of twelve hours, since sedimentation in a tube of this size is complete for most pathologic bloods by that time, whereas the nearer normal pathologic bloods are seldom complete, and the normal blood samples are never completed in a shorter period of time. I feel that it is very desirable to secure the entire sedimentation curve in order to evaluate properly the results of the test.

I have used 3.8 per cent aqueous sodium citrate as an anticoagulant because it is isotonic with the blood and gives a clearer plasma than heparin or the oxalates, which in the case of my technique is a distinct advantage.

A blue light source is used because, with the pale yellow media of the blood plasma and the opaque red cell volume, a more contrasting image is assured.

The technique of the test is as follows:

Four cubic centimeters of blood secured by venepuncture are mixed with 1 c.c. of 3.8 per cent aqueous sodium citrate in an ordinary test tube. The blood is pipetted to the black mark encircling the 4 mm. diameter sedimentation tube, which gives a column of blood 30 cm. long. A cork is placed in the bottom of the tube, and the tube is inserted into the machine. As the tube is placed in the machine, a switch is automatically closed; this starts the synchronous motor that rotates the drum, turns on the mercury vapor tube, and sets into operation the thermostatically controlled compartment. The test from then on is entirely automatic, including an automatic stop after twelve hours, until such convenient time after twelve hours that the operator may choose to remove the tube, take the machine into the darkroom, develop the record, and reload the machine to await the next test.

A simple and easy method of loading and unloading has been devised, and although our mechanism requires a darkroom for these two operations, which

are done in one sitting, I feel that inasmuch as the instrument is not heavy or bulky, the method is technically simpler than if photographic plates were used, because in either case a trip to the darkroom is necessary. The recording chamber is opened by lifting the dial knob, allowing the recording chamber end, which is hinged at the bottom, to swing open. The film mask is opened by disengaging the rubber bordered ends from their hooks, and the photographic paper is slipped under the mask. The chamber door is closed, and the dial is set at the arrow or "start line." The instrument is now ready to be used again.

The entire instrument consumes about 50 watts of electrical power. The synchronous electrical motor operating the drum makes one revolution in fifteen hours. The greatly reduced speed of the electrical motor makes it powerful and dependable. Twelve hours are used for the operating time, the space of the three remaining hours (6 cm.) being used for attachments of the mask and margins of the paper. The drum is 30 cm. in circumference; therefore, a point on the circumference of the drum moves 2 cm. each hour.

The size of the sedograph record, besides permitting attachment to hospital charts, lends itself well to being traced. A sheet of semitransparent tracing paper may be laid over the sedograph record and the curve traced thereon. Serial sedograph curves of a patient may be traced on this same sheet. It becomes a simple matter then for the physician to see at a glance a mechanically made, complete, and accurate sedimentation curve, as well as the most minute differences in serial readings.

I believe that the future will reveal the necessity of this degree of accuracy.

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A SEMISOLID DOUBLE SUGAR MEDIUM FOR THE IDENTIFICATION OF THE COLON-TYPHOID GROUP*

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RUSSELL'S double sugar medium has been used extensively in bacteriologic practice. By the use of this single medium, it is possible to obtain preliminary information of practical value in laboratory diagnosis. But its general application is limited, owing to the lack of differentiation between the motile *Eberthella typhi* and the nonmotile *Shigella* group, as well as between the motile *Salmonella* and the nonmotile para-colon group of organisms. Moreover, one of us has shown (P Y. L.)[1] that it is unnecessary to employ both lactose and dextrose in both the slant and the butt, because the purpose of differentiation can be satisfactorily accomplished by using dextrose alone in the butt and lactose alone in the slant.

Since 1938 we have employed a double sugar medium with a semisolid butt to indicate motility. Acid fuchsin has been used as an indicator since Cummings[2] has shown that this will give satisfactory results. Over 12,000 specimens of stool alone were examined in the past two years by this medium for preliminary identification. A total of over 2,000 positive cultures of various pathogenic gram-negative intestinal organisms was obtained. The following is the formula of this semisolid medium:

Solution A—Sugar-free broth (blood digest broth[3]), pH 7.4	100
Agar	0.35
Solution B—Sugar-free broth, pH 7.4	100
Agar	0.9
Lactose	1.0

Sterilize both solutions A and B by autoclave at 8 pounds pressure for fifteen minutes and cool to about 60° C.; add to both solutions A and B 0.5 c.c. of 2 per cent aqueous solution of acid fuchsin.

Keep solution B in water bath at 45° to 50° C. until used.

To solution A add 2 c.c. of a sterile 10 per cent solution of dextrose.

Fill tubes of 12 mm. inside diameter with 2 c.c. of solution A and cool in upright position until it solidifies. Then add to the same tubes solution B also in 2 c.c. amounts and let it solidify in a slanting position. The medium is now ready for use.

ACTUAL PROCEDURE AND INTERPRETATION

The stool specimens are, as usual, plated on the China blue rosolic acid medium, which in our hands have given satisfactory results. Next morning, after

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sixteen to twenty hours' incubation, each of several suspicious colonies picked from every plate is inoculated into a tube of this semisolid double sugar medium. After another twenty-four hours' incubation the results in this medium are read and interpreted according to the scheme in Table I.

TABLE I

RESULTS IN SEMISOLID MEDIUM				TENTATIVE DIAGNOSIS
Fermentation in		Production of		
Slant	Butt	Gas	Motility	
O	A	–	–	*Shigella*
O	A	–	+	*Eberthella*
O	A	+	+	*Salmonella*
O	A	+	–	Para-colon group
A	A	+	+ or –	Colon-aerogenous group
O	O	–	+	*Alkaligenes*

O = No change. – = Nonmotile or no gas production.
A = Red color. + = Motile or positive for gas.

For those cultures that show the first three types of reaction, further transfers into sugar fermentation tubes and agglutination with specific serum are made. Cultures showing the latter three types of reaction were discarded and considered to be those of nonpathogenic organisms. Care should, however, be exercised in cultures giving the type of reaction of the para-colon group, since the nonmotile *Salmonella* would give the same reaction. In such cases the amount of acid produced may give some indication, since the organism of the para-colon group usually produces a large amount of gas. If the amount of acid produced is slight, further studies in special sugars are indicated.

COMMENTS

The medium herewith reported makes use of the original principle of Russell's medium but has the following additional advantages:

(1) The consistency of the butt is sufficiently hard to stand up under the slant and at the same time is suitable for the demonstration of motility.

(2) The use of an inexpensive and stable indicator—acid fuchsin.

(3) The elimination of one of the sugars from both butt and slant, found to be unnecessary, effects material economy when large numbers of stool cultures have to be examined daily.

(4) Blood digest medium has been found to be sugar free and to support good growth for the organisms examined without the addition of peptone.

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DECREASED FRAGILITY OF ERYTHROCYTES INTERFERING WITH LEUCOCYTE COUNT*

MILTON G. BOHROD, M.D., MIAMI BEACH, FLA.

INCREASED resistance of erythrocytes to hypotonic solutions occurs infrequently. It is known to be a transitory phenomenon in hypochromic anemias, particularly after the loss of a large amount of blood, in some cases of obstructive jaundice and in pneumonia. The discovery of such decreased fragility requires the special technique of the commonly used fragility test. Whether cells more resistant than usual to hypotonic solutions are more resistant to other hemolytic agents has not been definitely determined. The following case is reported to illustrate the possibility of increased resistance of erythrocytes to acetic acid to the point where it interfered with the routine technique for counting leucocytes. The circumstances surrounding the discovery of this unusual phenomenon are as follows:

The patient was a 31-year-old woman, gravida iv, who had a breech extraction of a premature dead fetus (twenty-six weeks' gestation) on August 10. The placenta separated slowly and with difficulty, and there was considerable loss of blood. Her red blood cell count dropped in two days to 2,180,000, while she continued to pass blood clots from the vagina. In spite of transfusions on August 12 and 13, the erythrocyte count dropped to 1,820,000 on August 14. The color index varied between 1.01 and 1.05. Leucocyte counts were performed on August 12 and 13 (20,600 and 12,600), and nothing unusual was noted while doing these tests.

On August 14 the laboratory technician reported that she was unable to do a leucocyte count because of a large amount of what looked like debris in the counting chamber. Thinking she might have used a solution with precipitate of some kind in it or a dirty pipette, she attempted the count a second time with freshly made solutions; she obtained the same result. A third attempt still showed debris. In the meantime the solution had been used without difficulty on many other patients. The next morning, August 15, she was still unable to count the cells. At this time, examination of the debris suggested that it might be made up of clumped and distorted erythrocytes, and several rough tests were performed to test this hypothesis.

Blood was drawn from the vein, and enough sodium citrate was added to prevent clotting. A drop of this blood when added to the 1 per cent acetic acid usually used for leucocyte counts gave a rather muddy precipitate, and microscopic examination of this precipitate showed clumped red blood cells which could, however, be separated by pressure on the cover slip. A drop of blood was placed in 2 c.c. of 0.85 per cent sodium chloride solution. The red blood cells, for the most part, appeared perfectly normal microscopically, but a few of them were bowl shaped. A drop of blood was placed in 2 c.c. of distilled water. Many cells, of course, hemolyzed; but some cells did not hemolyze even after about fifteen minutes, and examination of these unhemolyzed cells showed nearly every one to be bowl shaped instead of the usual biconcave disc. Since this type of cell has been described[1] and shown to have decreased fragility or increased resistance to hypotonic solutions, it seemed possible that the cells in this case might also be abnormally resistant to acetic acid. Accordingly, attempts were made

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to count leucocytes using stronger solutions instead of 1 per cent acetic acid. With 2 per cent acetic acid a small amount of debris was still noted, enough to interfere with a reliable leucocyte count; with 3 per cent acetic acid used as diluting fluid, the field was clear and a leucocyte count could be made without difficulty. During the remainder of the patient's stay in the hospital white blood cell counts were made daily, using both 1 per cent acetic acid and 3 per cent acid as diluting fluids. On August 16, 3 per cent acid was required, but on August 17 and on succeeding days, it was possible to make the leucocyte count with the usual 1 per cent acid.

In the meantime the patient's erythrocyte count rose slowly, and on August 22 she was discharged from the hospital with a count of 3,200,000. The patient's blood type was type O.

Only a few additional tests were performed in the interval during which the increased resistance to acetic acid was demonstrable. The fragility test was inconclusive, for it was not read until twelve hours after its performance, and it is possible that if it had been read earlier it might have shown more striking results. On August 16 hemolysis was begun at 0.44 per cent sodium chloride and was complete at 0.32 per cent, a result not strikingly different from the normal and one which was not expected after the experiments with distilled water. Before the patient's discharge from the hospital a second test demonstrated hemolysis to begin at 0.46 per cent and to be complete at 0.36 per cent. Hence while the first test was at the lower limit of normal, there was a striking shift simultaneous with the change in resistance to acetic acid.

A galactose tolerance test was normal. The icterus index was 3, and cholesterol in the blood was 205 milligrams per cent.

The fragility of erythrocytes seems to be correlated with the shape of the cell. It is possible to devise a purely mechanical explanation which, at first glance, seems to fit the facts; but there seems to be some doubt as to whether this type of explanation is completely satisfactory. A spherical cell should be able to swell only slightly without disruption, and consequently will hemolyze in slightly hypotonic solutions. The normal biconcave disc can perhaps be conceived of as a partially collapsed cell and should have a greater capacity for taking up fluid than the spherical cell; this, of course, is exactly what happens. A bowl-shaped cell, such as that described by Barrett,[1] should have an even greater capacity for absorbed fluid and, therefore, a greater resistance to hypotonic solutions. The experiments of Barrett seem to show that these cells are resistant to 0.30 per cent sodium chloride and even lower concentrations, and my rough experiments with distilled water in this case seem to bear out this supposition.

The resistance of the cell to acetic acid, however, cannot be explained easily on this purely mechanical hypothesis and suggests that while the shape of the cell is definitely of importance, the physical and/or chemical structure of the cell also varies and is of importance. It is desirable that we know whether resistances to acid and to hypotonic solutions are parallel. The older work seems to indicate that resistance to hypotonic solution and to other hemolytic agents are parallel,[2] but Dameshek and his co-workers[3] suggest that there may be "differential fragilities." Mason and Rockwood[4] found fragility to any hemolytic agent to vary with the hydrogen-ion concentration. Furthermore, the possibility of the reversal of hemolysis,[5] as well as other experiments, suggests that rupture of a cell is not even a necessary part of hemolysis.

The bowl-shaped cell described assumes a peculiar form in dried smears, known as the target cell.[1] This is due to a characteristic indentation which takes

place during the drying of the cell, so that there is a central zone of hemoglobin-containing material, a midzone of little or no hemoglobin, and a peripheral zone again of hemoglobin. In our case, about 1 per cent of the cells in dried stained smears show this characteristic target appearance.

Inquiry among our technicians disclosed two previous instances in our laboratory in which it was not possible to perform the leucocyte count because of "debris." In both cases the phenomenon was transitory, lasting in one case one day, in the other, two days; but nothing was done to trace the cause of the phenomenon in these previous cases. The transitoriness of the increased resistance to acetic acid suggests that it may often be missed when repeated leucocyte counts are not performed.

SUMMARY

A case is reported in which, for a period of three days, it was not possible to perform a leucocyte count using the usual 1 per cent acetic acid as diluting fluid. It was, however, possible to make this count if 3 per cent acetic acid was used.

The cause for the difficulty in performing the leucocyte count was the marked increased resistance of the erythrocytes to acetic acid. The erythrocytes were also hyperresistant to hypotonic salt solution, and for a short time some of them were not hemolyzed even by distilled water.

The hyperresistant cells were bowl shaped in wet smears. Probably corresponding to these, target cells were noted in dry smears.

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420 LINCOLN ROAD

AN AID IN THE INJECTION OF THE EAR VEIN OF THE RABBIT*

E. E. Beohm, A.B., Philippi, W. Va.

VARIOUS means have been advocated for facilitating the injection of solutions into the ear veins of the rabbit, such as removing the hair, placing a paper clip on the ear, rubbing with xylol, and holding the rabbit in a box. All these procedures are of some help, but they leave much to be desired in certain cases.

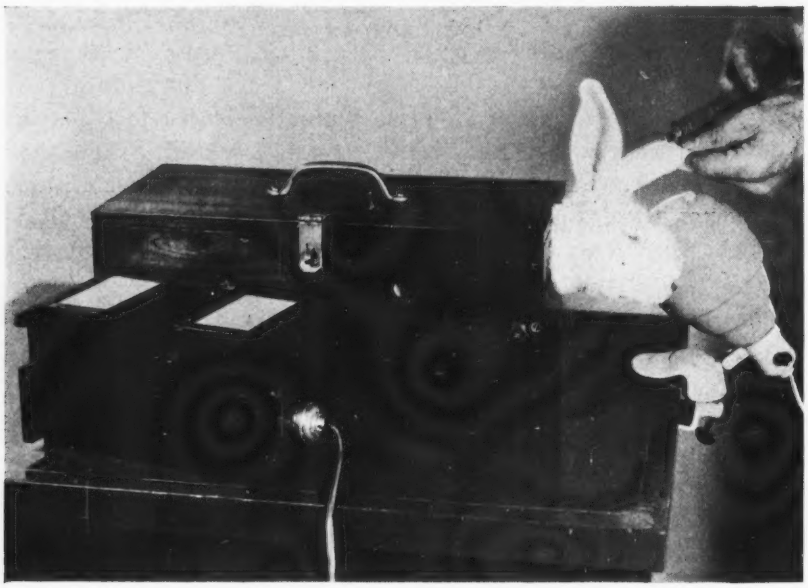

Fig. 1.

A simple arrangement that we have used in this laboratory for over three years, and that has proved very satisfactory, consists essentially in the use of a light placed below the rabbit's ear. All the veins stand out in view, so that one can be sure the needle is in, and by watching the blood being pushed back by the injected fluid can be certain that the solution all goes in the vein and not out into the tissues.

A light unit attached to the rabbit box makes a compact assemblage ready for use at any time. However, a spotlight bed lamp, which can be obtained at almost any hardware or department store, works very well. It can be clamped to the edge of the table so that the rabbit's ear can be held out over the lens. Fig. 1 shows this type of lamp in use, together with the light unit referred to above detached from the box.

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Some rabbits will remain quiet with little or no restraining and it is an easy matter to get into a vein and to stay in, but it is more particularly for those many other rabbits that are not so docile that this method is most useful. Also, where oft-repeated injections are done, the veins can be kept in better condition by the more easy and accurate venepunctures; furthermore, use is permitted of more and smaller veins.

When a rabbit moves his ears so vigorously that it is difficult to keep the needle in the vein, the needle can usually be kept in position by holding it and the ear of the rabbit together by pressure with the thumb on the hub of the needle against the fingers below the ear, and at the same time pulling slightly on the ear.

COMPOSITION TUBES AS A SUBSTITUTE FOR GLASS IN THE ISOLATION OF SPORULATING ANAEROBES

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EXPERIMENTS have been conducted for some time with the cultivation of sporulating anaerobes in tubes constructed of substances other than glass. Experiments thus far conducted seem to warrant a brief report, together with continued investigation.

The early experiments were conducted with small, spirally wound cellulose tubes, sold widely under the name of "Glassips." These were plugged with cotton at the upper end and sterilized in large glass tubes by baking at 160° C. for three hours. No extensive alteration of the tubes was effected by this heating.

Customary culture dilutions were made in melted tubes of suitable anaerobe agar. Each dilution was drawn up into separate pipettes ("Glassips") to a height of about 10 cm. The lower tip of each pipette was then dipped into a dish of sealing wax kept just above the melting point. These pipette cultures were then incubated at 37° C. as usual.

In these tubes we successfully cultivated *Cl. perfringens* and *Cl. tertium*. *Cl. sporogenes*, a much more strict anaerobe, grew only intermittently, and gave many failures. *Cl. botulinum* type A did not grow in any trial.

While this established the principle, it seems that these very thin-walled tubes do not maintain a sufficient degree of anaerobiosis to warrant their use for the purpose desired.

We next experimented with a larger and thicker-walled tube, sold commercially as a container for toothbrushes. This tube is of test tube construction, and is about 15 cm. in length and 2 cm. in diameter. It is composed, presumably, of some form of cellulose. This tube was plugged and baked. Melted whey agar was inoculated with *Cl. beijerinckii* and poured into the composition tube, hardened, then incubated at 37° C. Within twenty-four hours the colonies were visible to the naked eye.

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A control culture in a glass tube showed much more vigorous growth and extensive gas formation, while gas did not appear in the composition tube until after forty-eight hours. It was noted also that, in the composition tube, the colonies developed chiefly in the center of the agar column, not approaching closer than about 2 mm. from the wall of the tube. We presume from this either that the material in the wall of this composition tube is not an absolutely effective seal against oxygen, or that possibly some toxic agent may diffuse slightly from the wall into the medium. The former suggestion is the more probable explanation.

However this may be, the plastic tube served effectively to grow, in a deep agar column, an anaerobic organism which consistently offers difficulty in ordinary plate isolation.

The chief advantage of this proposed method of cultivation is that the colonies may be reached directly through the wall without cracking the tube, as is the usual procedure with glass tubes, or by slipping out the agar column, as is the case with the use of the Burri tube. Both of these methods offer opportunity for contamination unless the tube contains only a single species.

The wall of the composition tube may be washed with suitable disinfectant (it cannot be flamed), and a dissecting needle is heated to red heat. It is then easily and instantly inserted through the wall of the tube opposite the desired colony. A Pasteur pipette tip is heated and drawn to a fine capillary. This tip is snipped off and inserted through the hole into the colony. The semifluid colony contents can be observed to enter the capillary, which is then inserted into a tube of suitable sterile culture medium and tapped to break off the tip.

We are now seeking a variety of sizes of tubulatures of similar resin compounds, and hope to report more extensive experiments in the near future.* Horsfall[1] has recently reported the use of celluloid tubes for the preservation of frozen viruses. We believe that similar composition tubes may offer a new and effective means of anaerobic isolation, as applied especially to certain species which do not respond readily to the usual plating methods.

It may be added that this principle has been applied experimentally to plating, using the original Koch mica sheet cover idea, but substituting flat sheets of cellulose. We must admit that, thus far, the results are only imperfectly successful. Only *Cl. perfringens* and *Cl. tertium* have responded. We believe that thicker sheets of cellulose, together with greater depth of agar in the plates, may give more satisfactory results. At least the preliminary results appear to warrant continuation and modification of the experiments.

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*Since acceptance of this paper for publication, tubes and open tubing in a variety of plastic materials of suitable sizes and wall thicknesses have been obtained from the Dow Chemical Company, Tennessee Eastman Corporation, and the Lusteroid Container Corporation. The applicability of these to the problem of anaerobic isolation will be reported upon in a subsequent publication.

INEXPENSIVE SPECIFIC GRAVITY APPARATUS*

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DURING the past few years the determination of specific gravity by a more accurate technique, such as the falling-drop method of Barbour and Hamilton,[1] has increased tremendously in popularity. In many cases the specific gravity of the blood gives the essential information that much more time-consuming and costly albumin and globulin determinations convey. The method is simple and can be performed readily in a doctor's office in a few minutes, thus obtaining the results without delay and with very little inconvenience for the patient. In the medical school curriculum the student should be taught how to use this equipment, and the significance of such determinations with regard to blood, serum, urine, and body fluids. The chief drawback has been the expense involved in providing the high-priced manufactured outfits in sufficient numbers as routine physiology and pharmacology laboratory equipment for regular classroom use.

An inexpensive satisfactory specific gravity apparatus was devised, using a regular 2 liter unruled graduate cylinder as the container, for this holds sufficient water so that temperature does not fluctuate excessively. Two circular discs of sheet brass, $\frac{3}{64}$ inch thick, were cut to fit the graduate cylinder, with just enough play so that removal from the container was easy. Four holes were drilled into each disc (Fig. 1) for the glass tubes,† with a bore between 7.45 and 7.55 mm., as described by Barbour and Hamilton.[1] A central hole was drilled in each just large enough to allow a brass rod, $\frac{1}{4}$ inch in diameter, to pass through. One brass disc was then soldered flush with the end of the brass rod, and the other disc was soldered on the rod so that the distance was $2\frac{1}{2}$ inches from the top of the cylinder, when the first disc was placed on the bottom inside the container. The four holes in each disc were lined up in proper position at the time of soldering, one above the other, so that the glass tubes were held in a true vertical position. The central brass rod was cut off $1\frac{1}{2}$ inches above the upper disc and used as a handle, and the grip was improved by fitting a round nut on the end. Three legs were made from the brass rod and attached to the lower brass disc by drilling proper size holes as close to the outside edge as possible and soldering them in place. Each leg was made an inch long and spaced equally distant, one from the other. Two small holes were drilled in the upper disc, one on each side, between the holes for the glass tubes, each for a

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†The glass tube should be about 50 cm. long and the bore must be carefully checked at each end and fall within the prescribed dimensions. The tubes are marked with lines that extend all the way around exactly 30 cm. apart, which is the distance used in determining the drop falling time with a stop watch graduated in one-tenth seconds. The tube can be cleaned easier if the lower end is left unsealed and closed with a cork.

long stem centigrade thermometer. A small brass holder with a piece of brass placed through the open ring on the upper end was used to hold the thermometer in place (Fig. 2). Thus the thermometers were placed in a convenient position for reading and for accurate determinations of the temperature. Small brass rings, 1/16 inch wide, were placed around the glass tubes, containing the xylene-brombenzene mixture, exactly 30 cm. apart from inside edges. These

Fig. 1.

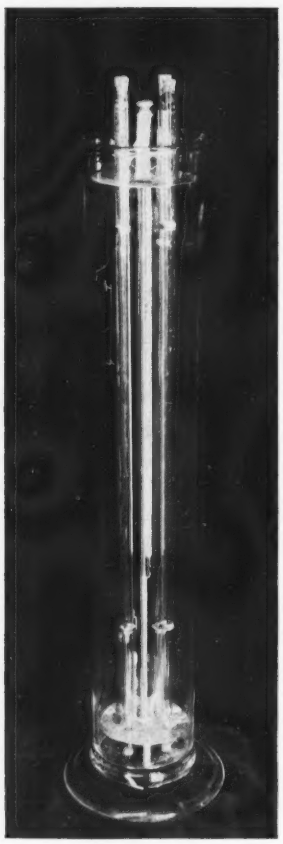

Fig. 2.

Fig. 1.—Photograph of stand for holding specific gravity tubes.
Fig. 2.—Photograph of stand in glass graduate containing specific gravity tubes in place.

increased the accuracy of stop watch readings of the drop falling time over plain scratch marks on the glass. All the apparatus placed in the glass graduate (see Fig. 2) can be removed together at once and quickly, which makes cleaning the tubes and refilling easy, or convenient for storing equipment. The rigid support holds the glass tubes firmly and securely in place. There are four tubes containing four different xylene-brombenzene mixtures, thus insuring an adequate range.

A one-handed controlled pipette holder, as described by Guthrie,[2] has been very satisfactory in this laboratory for use with the special 10 c.mm. pipette for measuring the size of the drop. A good technician can make a perfectly satisfactory holder at a very low cost.

The total cost of parts for this apparatus for the department at Columbia, Mo., was less than $2.00, exclusive of the labor, stop watch, thermometer, and pipette. The laboratory technician assembled the parts without difficulty. In case of breakage of the glass cylinder, the apparatus could be transferred to another quickly. Using the foregoing method for constructing the apparatus, one such piece of equipment can be supplied each four or five students without excessive cost, and accurate results can be secured for research as well as for teaching purposes. In many instances the specific gravity determinations training would be very valuable, for probably the specific gravity of the blood would be obtained in many cases which otherwise would be neglected if it were necessary to obtain total protein or albumin-globulin determinations.

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THE TECHNIQUE FOR THE ISOLATION OF BRUCELLA FROM HUMAN BRUCELLOSIS*

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INTRODUCTION

THE isolation of Brucella from human sources offers considerable difficulty. Various methods have been described,[1] all of which must be modified at times, depending upon the source of the material which is being cultured.[2-6] The procedures described here have been developed for routine use in the biologic division of Duke Hospital for the isolation of Brucella from blood, bile, lymph nodes, joint fluid, spinal fluid, feces, urine, and necropsy specimens.

METHODS

Blood.—Fifteen cubic centimeters of the patient's blood are introduced into a flask containing 4 c.c. of 2.5 per cent sodium citrate solution. Two cubic centimeters of the citrated blood are added to each of four deep tubes (15 c.c.) of molten liver infusion agar, cooled to 40° C. for pouring plates. Two cubic centimeters of blood are added to each of two flasks containing 100 c.c. of beef infusion broth, pH 7.4. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

All cultures are examined daily. After the first forty-eight hours' incubation, if no colonies are visible on the plates, 5 c.c. amounts of the supernatant broth are transferred from the flasks to sterile centrifuge tubes. After centrifugalization at high speed for fifteen minutes, the supernatant fluid is dis-

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carded, a loopful of the sediment is streaked over the surface of a liver infusion blood agar plate, and on a slide for a stained smear. The flasks are then shaken to mix the blood and broth, and 10 c.c. of this mixture are transferred to each of two flasks of fresh broth.

The colonies of Brucella in a poured plate containing blood resemble those of *Strep. viridans,* if such colonies are deep in the medium. After further incubation, especially if growing to the surface, the colony appearance resembles more closely that of *E. typhosa.* The colonies are picked and streaked over the surface of a liver infusion blood agar plate. The transplants are incubated under the same conditions as the original cultures. This procedure is repeated at two- to three-day intervals, until sufficient growth is obtained for identification. *Blood cultures for Brucella should be kept for eighteen days before being reported as negative.*

After there is sufficient growth, tubes of dextrose, lactose, maltose, mannite, sucrose, and plain beef infusion broth, pH 7.4, are inoculated. A nonmotile gram-negative coccobacillus, which ferments none of the carbohydrates and is agglutinated by antibrucella serum is diagnostic of Brucella. The bacteriostatic reaction of dyes[6] and agglutinin-absorption tests[7] are used to differentiate the species of Brucella.

These cultures may be supplemented by the inoculation of 2 c.c. amounts of the citrated blood into the peritoneum of each of two guinea pigs. *The animals are kept for three months if necessary before they are killed.* The spleen, liver, peritoneal fluid, and lymph nodes (if enlarged) of the animals are cultured for Brucella.

Bile From Duodenal Drainage.—The "B" bile is used for culture. One-tenth cubic centimeter of bile is added to each of four tubes containing 10 c.c. of beef infusion broth, pH 7.4, and the same amount of bile is streaked over the surface of each of two liver infusion blood agar plates. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

The cultures are examined daily after the first forty-eight hours' incubation. If the broth tubes are cloudy, 5 c.c. amounts are transferred to sterile centrifuge tubes. After centrifugalization at high speed for fifteen minutes, the supernatant fluid is discarded, and a loopful of the sediment is streaked over the surface of a liver infusion blood agar plate. A smear of the sediment is made and stained by Gram's method. If the broth tubes are not cloudy, 1 c.c. is transferred to tubes of fresh broth, and after forty-eight hours' incubation, the procedure just described is repeated.

On the streaked plates after twenty-four to seventy-two hours' incubation, the colonies are barely visible to the naked eye, appearing as minute, transparent colorless drops. *Microscopically, these are made up of small noncharacteristic gram-negative bodies, and one not familiar with the growth habits of Brucella may not identify this organism.* However, after transferring to fresh liver infusion blood agar and incubating for twenty-four to forty-eight hours, the colonies are larger and more numerous, and the organism is more typical in the Gram's stain. The transplants are incubated under the same condition as the original cultures. This procedure is repeated at two- to three-day intervals,

until there is sufficient growth for completing the identification. *The cultures should be kept for fourteen days before they are reported as negative.*

Lymph Nodes.—Lymph nodes removed by biopsy are cut immediately into small pieces with sterile scissors. Pieces of the node are streaked over the surface of two liver infusion blood agar, or North's gelatin blood agar, slants, or plates. One slant or plate is incubated in an atmosphere of 10 per cent carbon dioxide. If slants are used, the upper portion of the tube is heated in the flame to expel the air, which is then replaced by blowing the breath into the tube through a sterile plugged pipette, and inserting immediately a sterile rubber stopper. By this procedure a concentration of approximately 10 per cent carbon dioxide is obtained.

The cultures are examined daily. The first evidences of growth are tiny colonies resembling droplets of moisture, which may appear after forty-eight to seventy-two hours' incubation. *Stained smears from these colonies show minute coccoid bodies with indefinite outlines.* Transplants are made to fresh media every two to three days after the first seventy-two hours' incubation, *even though there may be no visible growth.* After one or two transplants the colonies are larger and stained smears show the usual coccoid or bacillary forms. The organism is identified in the usual manner. *The cultures are kept for three weeks before they are reported as negative.*

The remaining portion of the lymph node is ground in a sterile mortar, to which are added 2 c.c. of sterile physiologic saline. One cubic centimeter of this mixture is injected into the groin of each of two guinea pigs. The animals are followed in the same manner as those injected with blood.

Joint Fluid.—The fluid should be planted at the bedside, and it is essential that the medium used should be warmed to body temperature before it is used for inoculation. Two-tenths cubic centimeter of fluid is streaked over the surface of each of four liver infusion blood agar slants, and the same amount of fluid is put into each of two tubes containing 10 c.c. of beef infusion broth, pH 7.4. Two of the slants are incubated under increased carbon dioxide tension.

The cultures are examined daily, using the same routine as for lymph node cultures. *Cultures of joint fluid are kept for three weeks before they are reported as negative.*

Spinal Fluid.—One cubic centimeter of a 1:100 dilution of inactivated polyvalent antibrucella rabbit serum is mixed with 5 c.c. of spinal fluid and incubated at 37° C. for thirty minutes. After incubation the spinal fluid serum mixture is centrifuged at high speed for fifteen minutes, and 0.2 c.c. of the sediment is planted on each of four liver infusion blood agar slants, and into each of two tubes of beef infusion blood broth, pH 7.4. Two of the slants and 1 tube of broth are incubated under increased carbon dioxide tension.

The cultures are examined daily. The same routine as for lymph nodes is used for the blood agar slants, and the technique for the bile cultures is used for the broth cultures.

Feces.—About 1 Gm. of feces is mixed with 50 c.c. of sterile saline and shaken with a few sterile beads for five minutes. This suspension is filtered through four layers of sterile gauze into a sterile centrifuge tube. If the patient has agglutinins for Brucella in his blood, 0.5 c.c. of his inactivated serum

is added to the filtrate. Diagnostic serum without preservative is used if the patient does not have agglutinins. This mixture is placed in the icebox overnight, and the next morning it is centrifuged at second speed for two minutes. The supernatant fluid is discarded, the sediment is resuspended in 10 c.c. of sterile saline and recentrifuged. This procedure is repeated three times. After the last centrifuging, the sediment is resuspended in 5 c.c. of beef infusion broth, pH 7.4. A loopful of this mixture is streaked over the surface of four eosin methylene blue and two liver infusion blood agar plates. One cubic centimeter of the suspension is put into each of two flasks containing 100 c.c. of beef infusion broth, pH 7.4. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

The cultures are examined daily, after the first forty-eight hours' incubation. The colonies of Brucella resemble those of *S. dysenteriae* on the eosin methylene blue agar plates. These colonies are transferred to liver infusion blood agar slants. When there is sufficient growth, the organism is identified in the usual manner.

After the first forty-eight hours' incubation, the broth cultures are centrifuged, and the sediment is streaked over the surface of two liver infusion blood agar plates. Each of two flasks are inoculated with 1 c.c. of this sediment, and incubated for forty-eight hours, at which time the procedure is repeated. The transplants are incubated under the same condition as the original cultures. *Cultures of feces for Brucella should be kept for one week before they are reported as negative.*

Urine.—A fresh specimen of urine is centrifugalized at high speed for fifteen minutes. The supernatant fluid is discarded, and the sediment is used for culture. One-tenth cubic centimeter of the sediment is streaked over the surface of each of two liver infusion blood agar plates. One plate is incubated in the presence of 10 per cent carbon dioxide. When present, the organisms grow readily, producing typical colonies containing characteristic coccoid or bacillary forms. After forty-eight hours' incubation, if typical colonies are not present, transplants are made to blood agar plates at two- to three-day intervals. *The cultures are kept for ten days before they are reported as negative.*

Necropsy.—Cultures should be made as soon as possible after death. The surface of the liver, spleen, and kidneys is seared, and a small piece of tissue is removed with sterile scissors (a separate pair of scissors for each organ). If possible, axillary and mesentery lymph nodes are obtained. The small particles of tissue are washed through a series of three Petri dishes containing sterile saline, and then passed through the flame to remove any surface contaminants. The material is ground in a sterile mortar with sterile sand, to which are added 2 c.c. beef infusion broth. One-tenth cubic centimeter of this suspension is streaked over the surface of each of two liver infusion blood agar plates, and incubated in the usual manner. The lymph nodes are cultured as described previously.

The cultures are examined daily and transplants are made at two- to three-day intervals after the first forty-eight hours' incubation. *These cultures are kept for two weeks before they are reported as negative for Brucella.* Guinea pigs are inoculated and followed in the usual manner.

DISCUSSION

The cultures should be examined daily, and transplants of broth cultures should be made to fresh broth at two- to three-day intervals. A Gram's stain of the broth may not be very conclusive, unless the examiner is accustomed to looking at stained smears of the organism. When colonies on solid media first become visible to the naked eye, they appear as minute, transparent, colorless drops on the surface. *Transplants should be made to fresh agar at two- to three-day intervals even though there is no visible growth.* Brucella will not grow on dried agar, so, unless transplants are made to fresh, moist media, the colonies will not increase in size and the organism will disappear. When trying to isolate this group of organisms, it is very important to recognize growth when it first appears and to be able to distinguish between organisms and artifacts in the stained smear.

When organisms are present in small numbers, as in the blood of patients with the chronic form of brucellosis, and in lymph nodes, Brucella may be recovered occasionally from guinea pigs when the cultures are negative.

CONCLUSIONS

The cultural methods for the isolation of Brucella from various parts of the body are described in detail. Especial emphasis is laid upon the necessity of recognizing microscopically the very young forms of Brucella, and upon the study of the cultures for several weeks, if necessary. Disregard of these two factors may result in failure to demonstrate existing organisms.

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A NEW OBJECTIVE CIRCULATION TIME TEST (FLUORESCEIN METHOD)*

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A NEW method for estimating the speed of the circulating blood has been done on rabbits and on people. To date, the only reliable simple methods for determining complete circulation time are subjective in nature. A substance is injected into the antecubital vein of the arm, and the time is noted when the patient states that he tastes something bitter, burning, hot, etc., depending upon the substance used. Various substances have been suggested for determining the arm-to-tongue time. Decholin,[1] saccharin,[2] calcium gluconate,[3, 4] and magnesium sulfate[5] have been used. One must take into account inaccuracies in such a method. It cannot be used in small children, comatose people, or anesthetized patients. It would, therefore, be more accurate to have an objective method whereby a dye could be injected into the arm and the time noted when the dye appeared in the tongue, eye, etc.

I decided to use a very fluorescent substance, because even in high dilutions the color could be noted by the use of an ultraviolet machine with a filter (Woods filter). The ultraviolet machine was a water-cooled mercury quartz lamp (Kromayer lamp). Young male white rabbits, weighing 6 to 10 pounds, were used. With the rabbit's head projecting through an opening in a box, one assistant injected the fluorescent substance intravenously into the marginal vein of the ear, while another started the stop watch. Then the light switch was turned off, making the room completely dark. The light from the ultraviolet machine was directed toward one of the rabbit's eyes, and the conjunctiva and iris were noted to have a purplish hue. Suddenly a yellow color appeared on the inner margins of the eyelids (conjunctiva), and bright yellow vertical lines spreading into tiny branches were noted in the iris. This occurred simultaneously with a yellow color on the mucous membranes of the lips. There would thus be a sudden sharp end point to stop the stop watch. The time that elapsed between the rapid intravenous injection into the marginal vein of the ear, the passage of the substance through the right side of the heart, to the lungs, back to the left side of the heart, out through the aorta and to the periphery where it was best noted in the eye, was observed.

In rabbits I used the following fluorescent substances: 1 per cent acridine orange, 1 per cent flavophosphine, 1 per cent phosphine, 0.5 per cent acriflavine, 0.4 per cent indigo carmine, riboflavin, and 1, 5, and 10 per cent fluorescein.

RESULTS

2 c.c. 1 per cent acridine orange—Usually faint yellow reaction, but occasionally good yellow reaction.

1 c.c. 1 per cent flavophosphine—Poor yellow reaction.

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2 c.c. 1 per cent flavophosphine—Good yellow reaction.

2 c.c. 1 per cent phosphine—Fair yellow reaction.

2 c.c. 0.5 per cent acriflavine—Good yellow reaction.

2 c.c. 0.4 per cent indigo carmine—No color reaction.

2 c.c. riboflavin—Very faint yellow reaction.

2 c.c. 1 per cent fluorescein—Very good yellow reaction.

1 c.c. 5 per cent fluorescein—Very good yellow reaction.

1 c.c. 10 per cent fluorescein—Brilliant yellow reaction (best).

Therefore, fluorescein gave the best color reaction, being the most intense yellow when noted in the eye. Since sodium fluorescein is more soluble than fluorescein, the former was used to make up a 10 per cent solution. Acriflavine appeared to be the next best.

The circulation time test was done in over 70 instances, although the same rabbits were used on successive days or weeks. The same rabbit could even be used about one hour after the test, since much of the dye would disappear from the eye in that time. The circulation time in rabbits averaged between five and six seconds. Occasionally, it would be as low as 4⅖ seconds or as high as seven seconds.

In rabbits, 2 c.c. of 5 per cent sodium fluorescein injected intravenously gave a slight yellow reaction in the inner margins of the eyelids at the end of eight seconds, using ordinary daylight and no ultraviolet machine. However, the end point was not sharp. Therefore, for this experiment it is advisable to have an ultraviolet machine, a filter suited for fluorescence, and a room that can be made dark by turning off the light switch.

A 1 c.c. tuberculin syringe and a No. 24 gauge needle were used in the rabbits, since this was found to consume less than one-half second for intravenous injection.

The 10 per cent sodium fluorescein solution in distilled water was autoclaved for fifteen minutes, a sample was checked for sterility and toxicity by inoculation into a rabbit, and then used for intravenous injection in human beings. The circulation time was then noted to be 11⅘ seconds, 12 seconds, etc., in normal people. This was done in seven cases. There were no toxic reactions noted and no change in temperature, pulse rate, and respiration. A 5 c.c. syringe and a No. 21 or 18 gauge needle were used in man, with 4 c.c. of 10 per cent sodium fluorescein injected intravenously. In several additional cases, 3 c.c. of 20 per cent sodium fluorescein was used intravenously and gave even a better color reaction. There were no untoward reactions. By pulling the lower eyelid down and directing the ultraviolet light toward the conjunctiva after the substance was injected intravenously, a sharp yellow end point could be noted.

SUMMARY

A new objective circulation time test is presented, and after using various fluorescent substances, it was found that in rabbits the following technique was best. Using a 1 c.c. tuberculin syringe and a No. 24 gauge needle, 1 c.c. of 10 per cent sodium fluorescein was injected into the marginal vein of the ear. The

stop watch was started, the light was switched off in the room, the ultraviolet light plus filter was directed toward the rabbit's eye, and the stop watch was stopped immediately upon noting a brilliant yellow color in the iris of the eye. Simultaneously, the conjunctiva along the inner margin of the eyelids would become a brilliant yellow.

The average circulation time in rabbits was five to six seconds.

A similar test is being done in human beings, using 4 c.c. of 10 per cent sodium fluorescein or 3 c.c. of 20 per cent sodium fluorescein.

I wish to thank Doctors Follweiler, S. Guttman, I. Wolman, and Miss Spear for their valuable assistance.

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CHEMICAL

THE PHOTOMETRIC DETERMINATION OF SUGAR IN BIOLOGICAL FLUIDS BY FERRICYANIDE REDUCTION*

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WITH the advent of photometry or "photoelectric colorimetry" into biochemical analysis, numerous papers have been published on the photometric determination of sugar. Yet none of these methods has been so successful from the point of speed, precision, and reproducibility as to aid in supplanting the visual methods used at present. We feel that this paper meets such a need for the routine clinical analysis of sugar in biological fluids.

Photometric methods for sugar are in the main adaptations of existing copper or ferricyanide methods. Of the copper methods, modifications of the original Folin-Wu[1] have been the most popular. This method has been investigated by Sanford, Sheard, and Osterberg,[2] and by Fiorentino and Giannettasio.[3, 4] We have carefully investigated both the Folin-Wu and the Benedict[5] methods with a view to their possible adaptation to the photoelectric colorimeter. It was found impossible to obtain checks on runs performed from day to day, using the same set of reagents. Accurate results could be obtained only if a sufficient number of standards were run with each unknown, or set of unknowns, to allow for the plotting of a new calibration curve. An inherent advantage of ferricyanide methods is the greater stability of the reduction product, ferrocyanide, over the reduction products of the copper reagents. Therefore, photometric ferricyanide methods were then investigated. Hoffman[6] has described an adaptation of the ferricyanide fading method of Hawkins and Van Slyke.[7] The method has been found to give good results which are reproducible from day to day. The disadvantages of the method are: (1) an additional quantitative measurement is required, i.e., the ferricyanide in addition to the filtrate; (2) the spread is small, being only 30 divisions for a difference of 200 mg. of glucose per 100 c.c.; (3) the method is least sensitive at low sugar values and the most sensitive at high values.

The Folin-Malmros[8] ferricyanide colorimetric method has been found to be particularly suited for microsugar methods because of the intense blue colors obtained for small amounts of biological fluids. This method has been adapted for the photoelectric colorimeter by Jourdonais,[9] using the original procedure

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except for a longer heating time. This author finds that the colors produced do not follow Beer's law and give incomplete recoveries for added amounts of pure glucose. A special correction curve is required to obtain correct results. Marenzi and Villalonga[10] also have applied the Folin method to the photoelectric colorimeter. An advantage of their method is the substitution of lithium and ammonium tartrate in place of gum ghatti in the ferric iron reagent. The method, however, uses sodium cyanide in the alkaline reagent. (The undesirable effects of sodium cyanide will be noted later.) The method has also been investigated by Evelyn,[11] who increases the range of readings by leaving out the sodium cyanide from the carbonate reagent and runs his calibration curve in the presence of the tungstic acid solution used for the precipitation. The method still makes use of the ferric iron-gum ghatti reagent which gives variable blanks.

A visual method recently described by Klendshoj and Hubbard[13] does not use sodium cyanide, and substitutes for the gum ghatti an emulsifying agent (Duponol) which gives negligible blanks, yet stabilizes the Prussian blue solutions over long periods of time.

The last method, with several important modifications, has been adapted by us for purposes of photoelectric colorimetry. For routine sugar analyses the method has been found to give values that compare favorably in precision with the Hagedorn-Jensen[14] procedure and in speed of determination with the original Folin-Wu procedure.

EXPERIMENTAL

A. *Reagents.*—(1) Ferricyanide reagent: 0.1 per cent of pure, crystalline, potassium ferricyanide (prepared free of ferrocyanide according to Folin as given by Peters and Van Slyke[15]) was dissolved in a 3 per cent solution of sodium carbonate. The appropriate amount of ferricyanide was weighed on an analytical balance, made up to volume in a volumetric flask with 3 per cent sodium carbonate solution, and allowed to stand in a dark closet for several days. Any precipitate formed was removed by filtering the solution into a glass-stoppered, amber reagent bottle before using.

(2) Ferric iron reagent: Into a liter beaker weigh 5 Gm. of ferric sulfate (Mallinckrodt—analytical grade) and dissolve with stirring in about 500 ml. of distilled water. (Some difficulty may be encountered in bringing the ferric salt into solution, but HEAT MUST NOT BE USED if reproducible results are desired with each fresh batch of the reagent prepared.) Add slowly 75 ml. of 85 per cent phosphoric acid (reagent grade), stirring constantly. A precipitate or cloud forms, which quickly dissolves with the further addition of the phosphoric acid. An absolutely water-clear solution should now be obtained which is diluted to 1 liter with distilled water. Now add 16 Gm. of "Duponol" P.C. (an emulsifying agent sold by E. I. Du Pont De Nemours and Co., Fine Chemicals Division, New York, N. Y.) in small amounts, stirring constantly until complete solution takes place. Remove all traces of turbidity by allowing the solution to stand in an incubator at 37° C. overnight. Add 5 ml. of concentrated sulfuric acid (reagent grade, sp. gr. 1.84) to repress the hydrolysis of the ferric salt. The solution is now ready for use and will remain clear almost indefinitely if kept at room temperature when not in use.

(3) Tungstic acid* solution for the preparation of the Folin-Wu filtrate: Solution A: 6 ml. of concentrated sulfuric acid (reagent grade, sp. gr. 1.84)—diluted to 2 liters with distilled water in a volumetric flask. Solution B: 111 Gm. of sodium tungstate ($Na_2WO_4.2H_2O$) are dissolved in a liter of solution.

For use, 8 parts of solution A are mixed with 1 part of solution B. This should be prepared fresh at least once weekly.

For whole blood, serum, or plasma: one part of whole blood, etc., is mixed with 9 parts of the tungstic acid mixture. Shake. Allow to stand until the mixture turns chocolate brown, and filter through a Whatman No. 1 or No. 2 filter paper.

(4) Glucose standard solutions: (a) A solution of 100 mg. per 100 c.c. of pure, dry, anhydrous Bureau of Standards glucose was prepared in 0.25 per cent benzoic acid solution. (b) A solution of glucose containing 2 mg. per 100 c.c. was prepared by dilution of standard (a) with 0.25 per cent benzoic acid. 1 ml. is equivalent to 100 mg. of glucose per 100 c.c. (c) A solution of glucose containing 5 mg. per 100 c.c. was prepared as in solution (b). 1 ml. is equivalent to 250 mg. of glucose per 100 c.c. (d) A solution containing 10 mg. per 100 c.c. was prepared as in (b). 1 ml. is equivalent to 500 mg. of glucose per 100 c.c.

B. *Selection of Color Filter.*—The proper color filter was selected from spectrophotometric data, obtained over a range of glucose concentrations varying from 0 mg. to 400 mg. per 100 c.c., by means of a Coleman double monochromator spectrophotometer. Careful study of these data showed that Beer's law does *not* apply for any of the wave lengths studied. The most satisfactory transmission band was found to be in the neighborhood of 520 mμ. Six millimeters of a Sextant Green filter—Corning No. 401 (470 to 580 mμ transmission limits) were, therefore, used for all photometric determinations in this paper.

C. *Description of Instrument and Accessories.*—The instrument† used was essentially the same as that described by Evelyn[16] with several minor modifications so as to make it more suitable and convenient for the routine clinical analysis.

The diameter of the tube holder was made smaller so that it would accommodate ordinary pyrex test tubes (16 mm. by 150 mm.) instead of the larger soft glass tubes described by Evelyn. The tubes used in these determinations were carefully selected from large batches of pyrex tubes in the manner described by Evelyn[12] and were accurately calibrated for 5 ml. and 10 ml. volumes of solution. The determinations were performed directly in the tubes, and the colors were developed and read in the instrument *without* transfers.

The methods as described can be used without change with any standard photoelectric colorimeter now on the market that will accommodate a 10 ml. volume (or less) of solution. With minor adjustments in the volumes of the sample and the reagents used, the method is readily adaptable to any type of photoelectric instrument. For example, by using two and one-half times the

*Routine determinations for urea nitrogen are performed in this laboratory by adding a concentrated urease solution to the whole blood previous to the protein precipitation. A tungstic acid solution stronger than usual is required to precipitate both the excess urease and the blood proteins in order to obtain water-clear filtrates.

†The instrument was designed and built by Mr. Sidney X. Shore, Radio Engineer of Station WNYF, Fire Department of the City of New York.

quantities of sample and reagents given below, the procedure may be performed in regular Folin-Wu sugar tubes, with a final dilution to the 25 ml. mark. The color may then be read by means of transfer with photoelectric instruments which employ optical cells or ungraduated tubes.

Procedure.—(1) Preparation of the calibration curve: Two-tenths milliliter of a 1:4 dilution* of the tungstic acid mixture described above was pipetted into each of the pyrex photoelectric colorimeter tubes. Known amounts of the glucose standard solutions were then pipetted into the appropriate tubes using calibrated volumetric pipettes. One milliliter of the alkaline ferricyanide reagent was added with a 5 ml. serologic pipette to each tube. Distilled water was added to bring the total volume to about 4 ml. The tubes were heated in a boiling water bath for eight minutes, and then cooled in running water. Five milliliters of the ferric iron-Duponol reagent were then added and the contents were mixed. Distilled water was then added up to the 10 ml. calibration mark,†

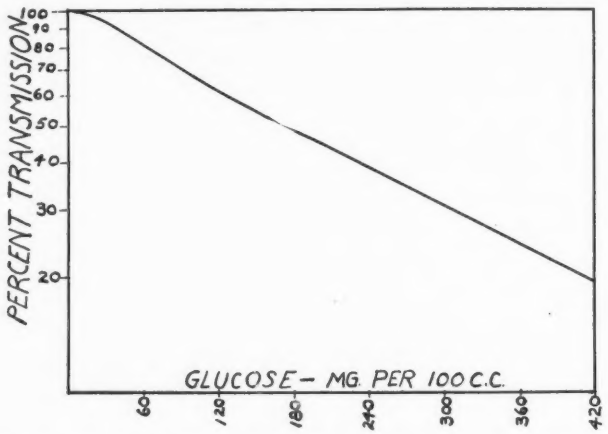

Fig. 1.—Calibration curve for standard glucose solutions for the colorimetric ferricyanide method. Corning filter No. 401-6 mm.

and the contents were mixed by inversion. The outside surface of the tubes was carefully washed and wiped dry with gauze or lens paper. The tubes were read in the instrument at various time intervals up to sixty minutes against a reagent blank set at 100 per cent transmission. The data for the thirty-minute interval were plotted on semi-logarithmic graph paper and are shown in Fig. 1. The reagent blank was constant and has a transmission of about 98 per cent against distilled water.

A summation of the data obtained indicates: (a) The curve follows Beer's law only between 100 mg. and 400 mg. per 100 c.c. of glucose with any degree of precision. Therefore, a calibration curve, or a table compiled from such a curve, is a necessity if accurate results are to be obtained. (b) Fluctuations obtained in the galvanometer readings for standard glucose solutions are greatest in values below 100 mg. per 100 c.c., and least in values of more than 100 mg. per

*The reason for the 1:4 dilution of the tungstic acid mixture will be discussed later in the paper.

†If foaming occurs, a wire dipped into caprylic alcohol may be touched to the surface of the solution in order to facilitate dilution to the mark.

100 c.c. (c) Good results can be obtained even for low sugar values if the readings are taken between thirty and forty minutes after the addition of the ferric iron reagent. (d) The checks obtained in the triplicate samples with the *same* glucose concentrations are excellent.

Readings on all further determinations in this paper were, therefore, made between thirty and forty minutes, and the calibration curve (or a table compiled from such a curve) was used to obtain the correct glucose values.

Analytical Determinations.—Hiller, Linder, and Van Slyke[17] have pointed out that the method of Hagedorn-Jensen can be applied to the determination of sugar in Folin-Wu tungstic acid blood filtrates with good results. If tungstic acid filtrate is used in the Hagedorn-Jensen method (in place of the zinc filtrate) from a theoretical point of view, the method should give results that are in perfect agreement with the colorimetric ferricyanide method we used. Both methods use a similar reducing reagent, i.e., ferricyanide in sodium carbonate solution, so that the reaction products formed in each of the two methods is the same. The only REAL difference between the two methods is that the Hagedorn-Jensen method determines the excess ferricyanide (which requires an additional quantitative measurement) by means of an iodometric titration using starch as the indicator, while our method determines the ferrocyanide formed directly by means of the Prussian blue color using a photoelectric colorimeter. Fujita and Iwatake[18] have found that the Hagedorn-Jensen method does not give strictly proportional reduction values over a wide range of glucose values, and for this reason they advocate the use of their own phosphate buffer. To offset this difficulty, since the original Hagedorn-Jensen was used in these determinations, the colorimetric determinations were always run first. Aliquots of filtrate were then selected so that the Hagedorn-Jensen values would all fall within a very limited range. As an added precaution, a set of blanks and a set of standards were run with each series of Hagedorn-Jensen titrations. With these added precautions, the two methods were found to give strictly comparable results.

(1) *Whole blood determinations:* Tungstic acid filtrate (1:10) of whole blood was prepared in the manner described under the preparation of the reagents. Two-tenths milliliter samples of the 1:10 filtrate or 2 ml. samples of 1:100 filtrate (where more accurate results are desired) were pipetted into the appropriate colorimeter tubes, using calibrated volumetric pipettes. One milliliter of the alkaline ferricyanide solution was then added, and the rest of the determination was carried out as given in the foregoing procedure. The tubes were read after standing for a thirty-minute interval. These determinations were run in triplicate for the purpose of comparison with the Hagedorn-Jensen procedure. A set of glucose standards was run with each set of unknowns. Recoveries were run on the whole blood samples by pooling many bloods together, so that a large quantity of blood was available. A sample of this pooled blood was taken off for analysis in the manner already described. An accurately weighed quantity of Bureau of Standards glucose was placed in a volumetric flask (100 ml.), the glucose was dissolved in the blood sample, and the solution was diluted to mark with the whole blood. Tungstic acid filtrates of this blood

were then prepared, and the sugar then present was determined. The difference between the two values obtained gave the recovery of pure glucose by this method.

The values obtained by this method for whole blood, their comparison with the Hagedorn-Jensen (also run in triplicate), and the recoveries obtained by this method are given in Table I.

TABLE I

DETERMINATION OF SUGAR IN BIOLOGICAL FLUIDS

(Every figure in table represents the average of three or more determinations.)

SAMPLE NO.	PHOTOMETRIC METHOD	HAGEDORN-JENSEN METHOD	GLUCOSE ADDED	GLUCOSE PHOTOMETRIC	
				CALCULATED	FOUND
	MILLIGRAMS PER 100 C.C.				
Whole blood					
1	221	214			
2	71	75			
3	100	102			
4	87	85	200	287	279
5	54	51	100	154	153
6	124	120	50	174	171
Plasma					
1	88	88			
2	48	49			
3	182	179	100	282	279
4	69	70	200	269	268
Serum					
1	54	56			
2	47	50			
3	49	50	100	149	148
4	62	60	200	262	265
Cerebrospinal fluid					
1	54	50			
2	128	133			
3	87	85			
4	64	63			
Urine					
1 (Normal)	0				
2 (Normal)	0		200	200	200
3 (Normal)	0		500	500	496
4	560	547			
5	1,250	1,220			

(2) *Plasma determinations:* Run as for whole blood. See Table I for the results obtained.

(3) *Serum determinations:* Run as for whole blood. See Table I for results obtained.

(4) *Spinal fluid determinations:* Determinations were performed directly on the spinal fluid samples without protein precipitation. The samples were diluted (1:6) with 0.85 per cent sodium chloride for the Hagedorn-Jensen determinations, and then these samples were diluted (1:10) again (or a total dilution of 1:60) for the colorimetric determinations. Because of the small amounts of material available, no recoveries were run. For routine analysis, 0.5 ml. of a 1:10 dilution of the original fluid may be used. The result obtained from the curve is then divided by 2.5 to obtain the correct glucose value.

The results obtained in these runs and their comparison with the Hagedorn-Jensen method are given in Table I.

(5) *Urine determinations:* The samples were prepared in the manner described in Peters and Van Slyke,[19] using Lloyd's reagent. A rough quantitative test was performed on each urine, and the sample was diluted in such a manner as to give values between 0.1 mg. and 0.2 mg. of sugar for the Hagedorn-Jensen runs, and 0.01 mg. and 0.02 mg. of sugar for the colorimetric determinations. Recoveries were run in the same manner as described under whole blood, using normal (or sugar-free) urine. The blank consisted of 2.0 ml. of a 1:1,000 dilution of this urine.

The results obtained in these runs and their comparison with the Hagedorn-Jensen method are given in Table I.

DISCUSSION

A careful physicochemical study of the reducing action of glucose on ferricyanide has been carried out by Wood,[20] using potentiometric measurements. We have investigated, by means of the photoelectric photometer, the effect of variations of concentrations of the reagents used in the above procedure (following the criteria laid down by Wood). In general, with one important exception (i.e., the use of sodium cyanide), our findings confirm those of Wood.

The concentrations of glucose used in this method (0 mg. to 0.08 mg. glucose or 0 mg. to 400 mg. per 100 c.c.) were chosen for the following reasons:

(a) A 0.2 ml. aliquot of a 1:10 Folin-Wu filtrate could be easily and accurately measured by means of calibrated mark to mark pipettes.

(b) It is *not* desirable to take readings below 20 per cent transmission, since the instrument is relatively insensitive in that region; i.e., large changes of glucose concentration produce relatively small changes of the transmission readings.

(c) The emulsifying agent (Duponol) can hold only a limited amount of Prussian blue in solution over long periods of time (minimum forty-five minutes). Concentrations of glucose greater than 400 mg. per 100 c.c. cause "cracking" or flocculation of the Prussian blue precipitate in relatively short periods of time.

The amount of potassium ferricyanide used in this method has been found to be sufficient to take care of the reducing action between 0.10 mg. and 0.12 mg. of glucose, i.e., 500 mg. to 600 mg. per 100 c.c. However, determinations of fluids with sugar concentrations of more than 400 mg. per 100 c.c. should be repeated, using smaller aliquots of the sample. The effect of the benzoic acid used to preserve the sugar standards caused no noticeable reduction of the ferricyanide. In order to duplicate as closely as possible the conditions under which glucose is reduced in a blood filtrate, the average concentration of tungstic acid remaining in blood filtrate after precipitation of the proteins was determined gravimetrically.[21] This quantity of tungstic acid was then added to the glucose standards. It was noted that the alkalinity of the ferricyanide affects the rate and amount of reduction considerably. Experimental results obtained showed that a ferricyanide reagent containing 3 Gm. of sodium carbonate per 100 ml. of ferricyanide solution (0.1 per cent) gave optimum results. Experimental runs made to find the minimum time of heating showed that at least five minutes was necessary for concentrations of sugar below 400 mg. per 100 c.c. Therefore, a

heating time of eight minutes was selected to give a safe margin of time for the complete reduction of the ferricyanide by glucose. The use of sodium cyanide, as used in the original Folin reagent, was found to be undesirable, because it reduces the range of the method by one-half, and it also seriously affects the reproducibility of results for the reason that the amount of ferrocyanide produced increases with the time of heating up to about forty-five minutes when it begins to level off.

That precise and reliable results can be obtained by this method is shown by the analytical data given in this paper for the various biological fluids. Results obtained by the photometric method are in close agreement with those of the Hagedorn-Jensen method, and the recoveries obtained for added glucose are excellent. The method, as given here, has been used in this laboratory for routine clinical analysis for more than one year with uniformly excellent results. Blanks, and 100 mg. and 200 mg. per 100 c.c. standard glucose solutions have been run daily for that period, with an average error of about ±2 per cent. In the course of performing these routine determinations and doing the analytical work of this paper, new batches of each reagent were made up at relatively frequent intervals. We were able to check constantly the points of our original calibration curve.

The method, in addition to being more precise, reliable, and economical of material than the old Folin-Wu procedure proved to be much faster for a large number of sugar determinations. The only quantitative measurement required is that of the 0.2 ml. of the 1:10 Folin-Wu filtrate. The sodium carbonate-ferricyanide solution is pipetted as one reagent, and all the succeeding operations are performed in the individual pyrex tubes without any transfers. The colors developed are read in these same tubes at the rate of about five a minute.

Some objections to the method may arise from the fact that a long waiting time (thirty minutes) is required to allow for full color development before the colorimeter readings are taken. This would be of particular importance in those laboratories where only a few determinations are performed at one time, or in the case of an emergency determination. For emergency determinations, results of sufficient accuracy may be obtained by taking the readings at a ten or twenty minute-time interval, or if more accurate results are desired, additional calibration curves may be drawn for these time intervals.

In agreement with Somogyi[22] we have found that ferricyanide methods give sugar values that average about 20 mg. per 100 c.c. higher for tungstic acid filtrate than those obtained by the copper methods of Shaffer-Hartmann[23] or Benedict. These copper methods give "true sugar" values in that they are not affected by the nonglucose reducing substances present in the tungstic acid filtrate.

We have confirmed the observations of Hoffman, and of Klendshoj and Hubbard, that the colorimetric ferricyanide method gives results which are in agreement with those obtained by the old Folin-Wu procedure. The normal values for sugar obtained by this method are practically the same as those given by the old Folin-Wu procedure, i.e., from 85 mg. to 115 mg. per 100 c.c. of blood sugar.

SUMMARY

A photometric ferricyanide method is described for the rapid, precise determination of sugar in small amounts of biological fluids, e.g., 0.2 ml. of a (1:10) blood filtrate. Careful studies of the factors influencing the reduction of ferricyanide by glucose were made in order to determine optimum conditions and concentrations of reagents. Negligible and constant blanks were obtained by the substitution of "Duponol" for gum ghatti. Reproducible values for standard solutions were obtained for day to day runs even when new reagents were used. The methods gave results for biological fluids that were in agreement with those obtained by the Hagedorn-Jensen procedure and gave excellent recoveries of added glucose.

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THE DETERMINATION OF BLOOD LIPIDS IN BLOOD DYSCRASIAS*

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THE objects of this paper are to compare the values for total blood plasma lipids in blood dyscrasias obtained from the usual alcohol-ether extracts and from petroleum-ether extracts, and to study the nature of the nonlipid contaminants in these extracts.

Pernokis and Freeland[1, 2] reported elevated total lipid values obtained gravimetrically from alcohol-ether extracts in cases of blood dyscrasias. Their work was well in progress when Christensen[3] and almost simultaneously, Van Slyke and Folch,[4] reported that nonlipid contaminants were present in the alcohol-ether extracts and also, to a lesser degree, in the petroleum-ether extracts. They also clearly demonstrated that the bulk of these contaminants were urea and chlorides.

We approached the problem in two ways which will be described in turn. The first method was to compare the total lipid values obtained from alcohol-ether extracts with those obtained from petroleum-ether extracts.

Blood plasma was extracted with the usual alcohol-ether mixture. Aliquots of this extract were carefully evaporated to dryness and weighed. This value appears in Table I as "Total Lipids" under the heading "Alcohol-Ether Extract." This alcohol-ether soluble residue was then extracted five times with 5 c.c. portions of petroleum-ether extract. The combined petroleum-ether extract was then carefully evaporated to dryness and weighed. This value appears in Table I as "Total Lipids" under the heading "Petroleum-Ether Extract."

To obtain the values for "Total N as Urea" in Table I, aliquots of both the alcohol-ether and petroleum-ether extracts were subjected to micro-Kjeldahl analysis. The values obtained for total nitrogen were converted to their equivalents in terms of urea because urea is the form in which practically all of this nitrogen is present. The values for "Corrected Total Lipids" in Table I are the values obtained by subtracting the "urea" values from the "Total Lipids" values.

A comparison of the values for "Corrected Total Lipids," as shown in Table I, reveals that only from 46 to 72 per cent of the materials soluble in the alcohol-ether mixture from cases of myelogenous leucemia are soluble in petroleum-ether.

The second method of approach to the problem was an attempt to determine the composition of the material, which is soluble in the alcohol-ether mixture but insoluble in pretroleum-ether extract. The procedure used was to use mixed

*The material for this paper was obtained at the Cook County Hospital, Chicago. The determinations were carried out in Laboratory C of Cook County Hospital and the Laboratories of the Presbyterian Hospital, Chicago.

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alcohol-ether extracts from blood dyscrasias. The alcohol-ether mixture was carefully evaporated and the dry residue was weighed. The weight of this alcohol-ether soluble material was 0.1605 Gm. It was then extracted five times with 5 c.c. portions of petroleum ether. The petroleum ether was carefully evaporated. The dry residue weighed 0.0733 Gm. This is only 45.7 per cent of the weight of the alcohol-ether soluble substances. The petroleum-ether insoluble material (0.0872 Gm.) was dissolved in 100 c.c. of distilled water. This aqueous solution was used to determine chlorides as sodium chloride, urea, uric acid, creatinine, and total reducing sugars. The results are shown in Table II.

TABLE I

DIAGNOSIS	ALCOHOL-ETHER EXTRACT			PETROLEUM-ETHER EXTRACT			
	TOTAL LIPIDS (GM.)	TOTAL N AS UREA PER 100 C.C.	CORRECTED TOTAL LIPIDS PLASMA	TOTAL LIPIDS (GM.)	TOTAL N AS UREA PER 100 C.C.	CORRECTED TOTAL LIPIDS PLASMA	% PETROLEUM ETHER SOLUBLE
1. Pernicious anemia	1.270	0.053	1.217	0.856	0.021	0.835	68.6
2. Pernicious anemia	1.153	0.040	1.113	0.680	0.027	0.653	58.6
3. Acute myelogenous leucemia	1.293	0.058	1.235	0.933	0.044	0.889	71.9
4. Chronic myelogenous leucemia	1.400	0.048	1.352	0.666	0.043	0.623	46.0
5. Cholesterolemia	4.733	0.114	4.588	4.460	0.088	4.372	95.4
6. Leucemia cutis	1.400	0.072	1.327	0.800	0.034	0.766	57.7
7. Lymphatic leucemia	1.466	0.050	1.416	Accidentally lost			---
8. Uremia	2.133	0.167	1.966	1.130	0.157	0.973	49.4
9. Lymphatic leucemia	1.353	0.050	1.303	0.633	0.026	0.607	46.5
10. Chronic myelogenous leucemia	1.300	0.077	1.223	0.766	0.028	0.738	60.3

TABLE II

Sodium chloride	53.00 mg.	or 60.8 per cent of residue
Uric acid	0.25 mg.	or 0.3 per cent of residue
Creatinine	0.42 mg.	or 0.5 per cent of residue
Urea	33.00 mg.	or 37.8 per cent of residue
Reducing sugars	0.00 mg.	
Total accounted for	86.67 mg.	or 99.4 per cent of residue
Amount insoluble in petroleum ether		0.0872 Gm.
Amount recovered		0.0867 Gm.
Amount unaccounted for		0.0005 Gm.

The petroleum-ether insoluble contaminants consisted of 60.8 per cent of chlorides calculated as sodium chloride and 37.8 per cent of urea. These two contaminants accounted for 98.6 per cent of the total. Uric acid and creatinine accounted for 0.8 per cent, leaving 0.6 per cent unaccounted for. No reducing sugars were demonstrated to be present.

The petroleum-ether soluble material (0.0733 Gm.) was redissolved in petroleum ether and washed five times with 5 c.c. portions of water. The petroleum-ether was carefully evaporated, and the residue was reweighed. The loss of weight was 0.0087 Gm. of material dissolved in the water. This represents a loss of 11.8 per cent of the total petroleum-ether soluble material. The complete nature of this material has not yet been determined, but some of it is urea and chlorides. The problem is being further investigated.

DISCUSSION

The total corrected petroleum-ether soluble lipid values in two patients with pernicious anemia were 69 and 59 per cent of the values obtained for the alcohol-ether extract. In three patients with myelogenous leucemia, the total corrected petroleum-ether soluble lipid values ranged from 46 to 72 per cent of the values obtained for the alcohol-ether extract. The corrected petroleum-ether extraction values in three persons with lymphatic leucemia were 46 to 58 per cent of the alcohol-ether values. In one patient with uremia the petroleum-ether soluble material showed 49 per cent of the value obtained with the alcohol-ether extraction. The most interesting results were obtained in one person with hypercholesterolemia which showed a very marked increase in the total lipids of the blood with the alcohol-ether extraction method, and continued to show 95 per cent of that value in the petroleum-ether extract. This patient showed a tremendous increase in the fatty acids of the blood, values being as high as 2.758 Gm. Due to the unusual character of the lipids in this blood, the results were checked by three different chemists and found to agree.

CONCLUSION

Total lipid determinations done with the alcohol-ether extraction method show values which are much higher than the values obtained with the petroleum-ether extraction method. The values obtained with the petroleum-ether extraction varied from 46 to 95 per cent, with an average of 60.6 per cent of the total lipid values obtained with the alcohol-ether extraction.

The petroleum-ether insoluble material extracted with the lipids in the alcohol-ether extraction was analyzed and showed to contain 61 per cent of sodium chloride, and 38 per cent of urea. The other 1 per cent was present in the form of uric acid and creatinine. No reducing sugars were found.

Washing the petroleum-ether soluble material five times with distilled water showed a loss of 8 mg. of material by weight, which is 11.8 per cent of the total petroleum-ether soluble material.

The one patient with hypercholesterolemia did not show a marked difference in lipid values with the two methods of extraction.

On the basis of all the total lipid values given for normals, as done by the alcohol-ether extraction method by all the accepted authorities, our values for the blood dyscrasias have been high. Our values in the above series with the petroleum-ether extracts average 60.6 per cent of the values obtained with the alcohol-ether extracts, and are higher than normal.

It is obvious on the basis of the findings of Christensen, and Van Slyke and Folch, whose findings we now confirm, that a new standard of normal total blood lipid values must be determined. We are now investigating this question and we shall report our observations on it later.

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MEDICAL ILLUSTRATION

RUBBER CASTS OF MEDICAL SUBJECTS

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WAX formulas have been used for some time for casting lifelike subjects. Although they give a true representation of the subject, they are not as durable as might be desired. By substituting prevulcanized latex for the wax, a permanent cast may be obtained. Such rubber casts have many of the advantages of the wax cast, with few of the disadvantages. Fig. 1*A* is a photograph of a spleen chosen to illustrate this process. The subject is a poor one, since it was removed from a body that had been preserved for several months. However, it is adequate for this description. A fresh specimen would have been more desirable.

Before starting the actual molding, a cardboard or glass box is made to hold the model. Glass was used for the box seen in Fig. 1*B* and serves to illustrate better the process. A small amount of plaster is poured into the center of the box. The specimen is placed in the plaster and vibrated to rid the surface of bubbles. By pounding several times on the table near the box, these bubbles will disappear, provided the plaster is in a liquid state. Additional plaster is poured into the box and a brush is used to work the plaster up to the edge of the specimen (Fig. 1*C*). This edge serves as the line of separation previously determined.

After the plaster is set, it is removed from the box, and the spleen is then dislodged from the set plaster. The edges around the impression of the spleen are trimmed of loose plaster and particles that are likely to break off. This set plaster form constitutes the lower half of the mold. Registration grooves are then cut in each corner, as seen in Fig. 1*D*. These are necessary to insure a perfect fit of the upper and lower halves. The edges of the lower half are greased with petroleum jelly so that it will separate readily from the upper half which is to be made, as shown in Fig. 1*E*. Care must be taken to prevent the petroleum jelly from getting on the surface of the plaster created by the spleen. If this happens, the surface ceases to absorb the water from the rubber which is to be poured later. The specimen is then replaced.

The plaster is mixed and the upper half of the mold is made, as shown in Fig. 1*F*. The molding material is applied with a brush into the crevices on the surface of the spleen. The procedure previously described should be followed to remove any air bubbles that may be present. After the plaster has set, the mold is opened and the specimen is removed. There are now two halves of the mold. Before these are put together for the making of the rubber positive,

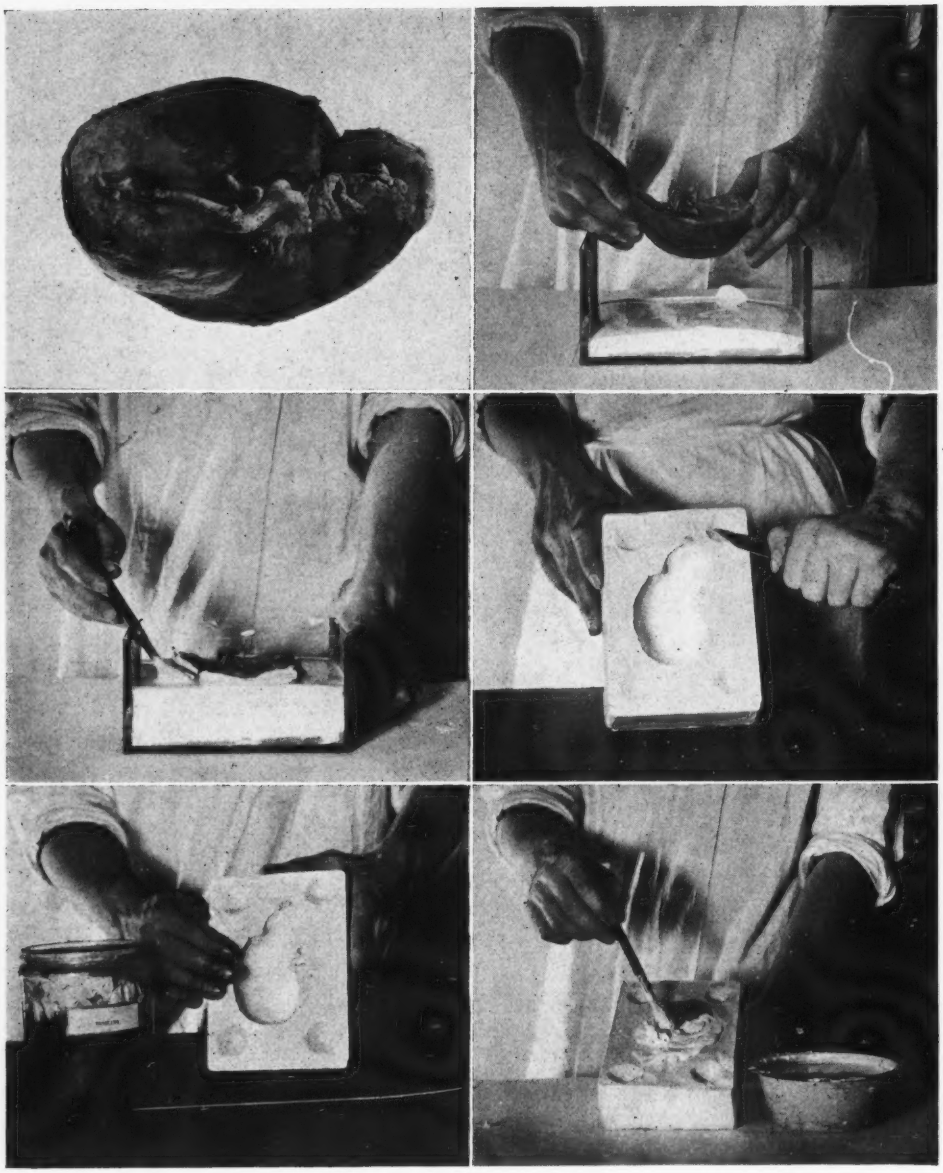


Fig. 1.—*A*, A preserved spleen.
B, The spleen is placed in a glass box containing a small amount of plaster.
C, The plaster is worked up to the edge of separation which has been determined previously.
D, Registration notches are cut in the set plaster.
E, Petroleum jelly is applied to the edge of the first half of the mold.
F, The specimen is replaced and the plaster applied to form the second half of the mold.

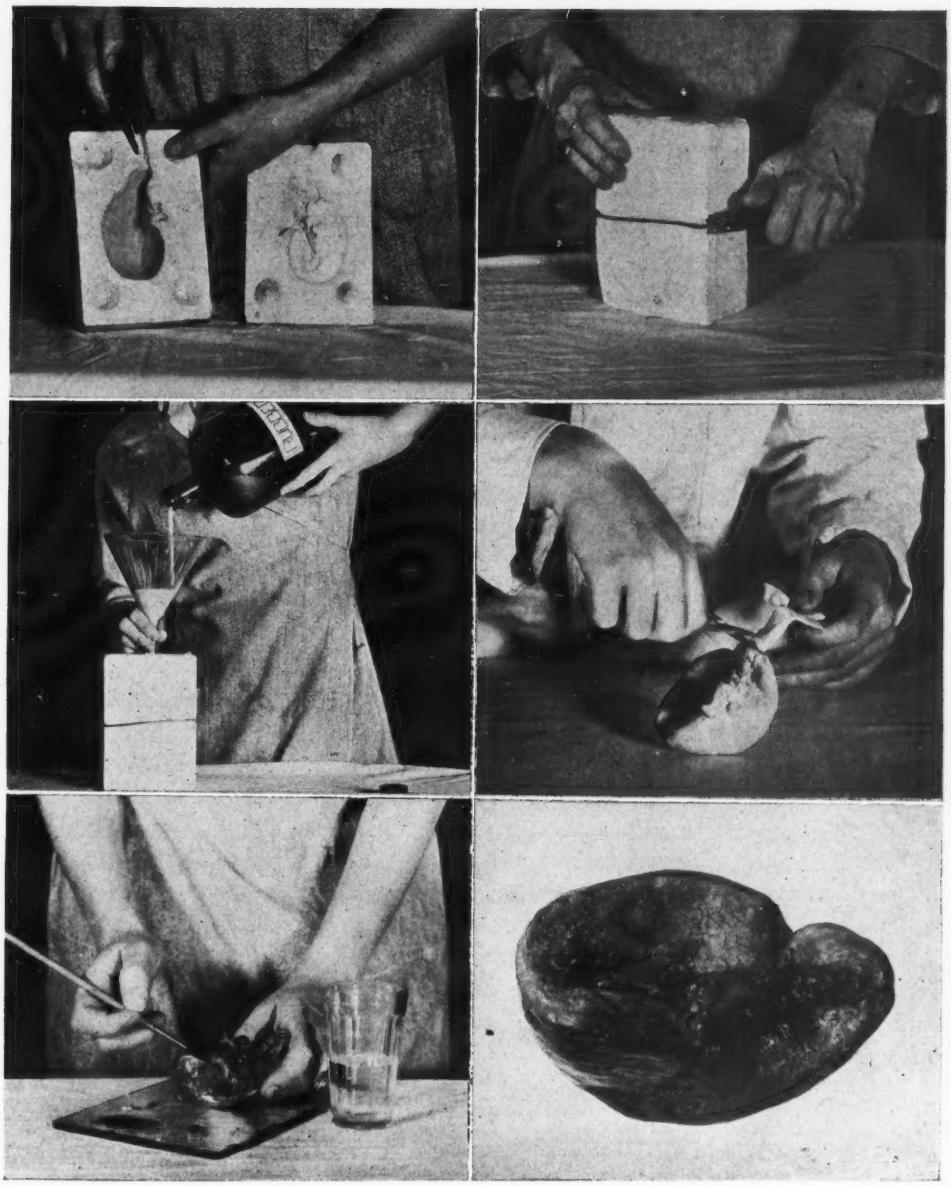


Fig. 2.—*A*, An opening is provided into which liquid rubber may be poured.
B, The two halves of a mold are held together with wire or rubber bands.
C, Liquid rubber is poured into the closed mold. After sufficient thickness is built up, the excess rubber is poured out.
D, The rubber formed by the funnel-shaped opening is cut away.
E, The specimen is colored to resemble a fresh spleen.
F, The completed cast.

a funnel-shaped opening should be cut at the end that affords the best drainage, as illustrated in Fig. 2*A*. The two halves are placed together and held securely with wire or large rubber bands (Fig. 2*B*). Such rubber bands can be cut from an old inner tube and serve well for this purpose. The mold is then allowed to dry thoroughly. This usually takes several days; if gentle heat is applied, only a few hours are required to dry the mold.

Liquid vulcanized latex, which has been mixed with a mineral filler such as zinc oxide, is poured into the mold until it is filled completely (Fig. 2*C*). The mold is then allowed to set until the desired thickness is built up on the inside. As the dry plaster absorbs the water from the liquid rubber, more rubber is added to keep the mold filled at all times. When the cast has obtained the desired thickness, the excess rubber is poured out. This deposit is developed as a crust next to the plaster mold. The thickness of the crust depends on the dryness of the plaster and the consistency of the rubber-filler mixture. For those persons familiar with casting in terra cotta with clay slips, it should be mentioned that the rubber cast is developed within the plaster mold in a similar manner.

When the desired thickness of the rubber has been built up within the mold and the excess latex has been removed, the cast within the mold is allowed to dry overnight. Gentle heat speeds up this process and at the same time dries the plaster mold, thus making it more suitable for casting a second impression. The mold is then opened and the cast is removed. The part created by the funnel-shaped opening is cut away, as seen in Fig. 2*D*. Unless a nonmetallic water color, having a base reaction, is added to the liquid rubber before it is poured, the resultant cast will be cream colored. Any material having an acid reaction causes the rubber to coagulate and makes it unfit for use. Furthermore, rubber should not be mixed in bowls or containers made of copper or zinc, since these metals destroy the elasticity of the rubber and prevent it from setting properly. After the cast has set within the mold, it is removed. Any trimming or correction of defects that may be necessary should be done with fine-pointed scissors, a razor blade, or a sharp knife that has been dipped into water. The water causes these instruments to cut the rubber without dragging or leaving any rough spots. Small defects are removed with sandpaper, and the cast is given a coat of white shellac or lacquer to prepare it for coloring.

The transparent oil colors used for photo tinting serve excellently for coloring such casts, as illustrated in Fig. 2*E*. After the color is dry, the cast is given a coat of dammar varnish to make it resemble more closely the wet fresh specimen.

It is absolutely necessary that the color be dry before applying the varnish. Fig. 2*F* shows the completed rubber spleen. This may be compared with the original seen in Fig. 1*A*.

The specimen can be mounted in various ways. A base may be attached to the specimen at the point of the funnel-shaped opening. A thin metal rod is generally forced through this opening and sealed within the cast. The other end of the rod is anchored through a wooden or cast metal base.

The liquid vulcanized latex used for this purpose may be purchased from the General Latex and Chemical Co., Cambridge, Mass.; which firm sells the rubber already mixed with the filler. The latex may also be bought for mixing with any filler for specific purposes. This company is not interested in small orders and refers the prospective buyer to one of its agents who sells the vulcanized latex or one of the mixtures under a trade name at higher prices. In gallon lots the price should not exceed fifty cents a pint. However, it is sold under various trade names for as much as three dollars a pint. The average completed specimen would contain only three or four ounces of rubber. However, as much as two quarts may be necessary to fill the mold in order to get the four ounce deposit. The rubber which is poured from the mold may be used again.

The technical process is slightly different with each specimen that presents its own problems. For example, openings, air vents, and drainage vents must be placed at different points, according to the specimen that is being molded. Therefore, this description will overlap at points and be repeated.

The heart is a more complicated subject. A fresh specimen is easier to work with and gives better results than the preserved specimen used in this case. The wall to confine the plaster is made of cardboard (Fig. 3*A*). The plaster is poured into this space and worked up to the line of separation which has been determined previously. After the plaster has set and formed half of the mold, the specimen is removed (Fig. 3*B*). The edges are then trimmed and registration grooves are cut. This is done so that the other half of the mold, which is to be made, will fit perfectly with the first half.

After the registration grooves are cut, the first half of the mold is greased with petroleum jelly (Fig. 3*C*) to make separation possible. Care must be taken to prevent the petroleum jelly from touching the area to be covered with rubber, since the jelly prevents absorption of the water from the rubber into the plaster.

The specimen is then replaced in the mold, and the other half is made (Fig. 3*D*) by applying the plaster-water mixture directly to the specimen, thus covering the specimen and the first half of the mold.

In Fig. 3*E* are shown the two halves of the mold with the specimen incased. On one-half of the mold an area is outlined in pencil to form the entrance for a funnel through which the rubber is to be poured (Fig. 3*F*). Notice that this penciled area extends into two parts of the specimen, one at the base of the heart and the other at the cut section of the descending aorta. This allows the liquid rubber to drain from both parts of the specimen. The penciled area is cut out of the plaster to permit the insertion of the funnel seen in Fig. 4*C*. These areas also serve to drain the rubber from the mold in creating a hollow cast; therefore, they are cut into areas that are likely to form pockets. Pockets without openings will prevent the rubber from flowing out by gravity.

The two halves of the mold are wired together or held together with large rubber bands cut from sections of old automobile inner tubes (Fig. 4*B*). After the mold is thoroughly dry, the liquid vulcanized latex is poured through the

opening (Fig. 4*C*). The mold is then allowed to set until a sufficient thickness is built up by the absorption of the water from the liquid rubber into the mold. If the plaster is thoroughly dry, this takes only a few minutes; if the mold is wet, it may take hours. After a cast of sufficient thickness has been formed, the excess rubber is poured out and the hollow specimen is allowed to set overnight to strengthen the rubber. Gentle heat hastens this process and has a

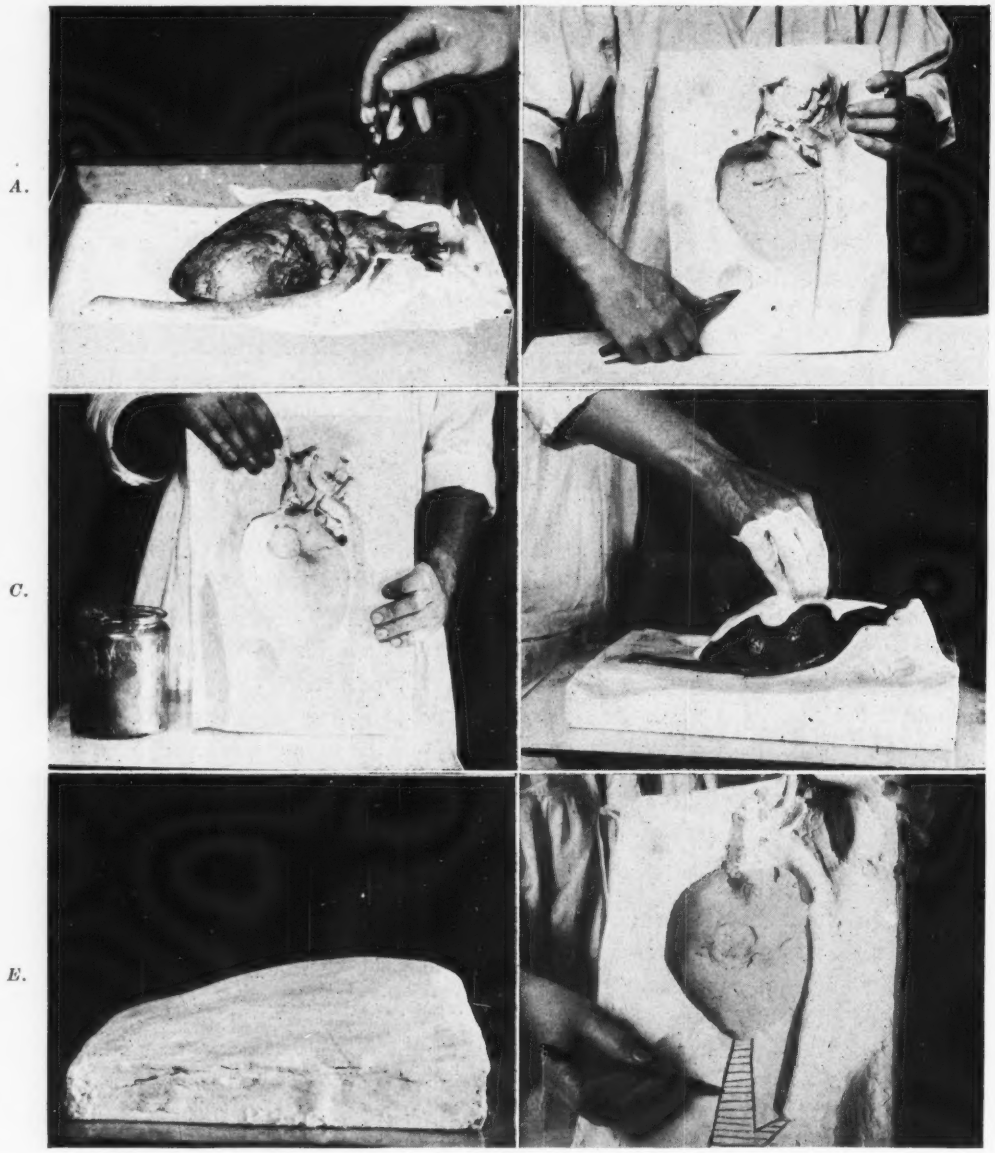


Fig. 3.—*A*, Making the first half of the mold.
B, Cutting registration grooves.
C, Petroleum jelly applied as a separating medium.
D, Making the second half of the mold.
E, The two halves of the mold with the specimen incased.
F, Marking the area to be cut away for pouring the rubber.

beneficial effect on both the mold and the cast. When the specimen is thoroughly dry, the mold is opened and the cast is removed (Fig. 4*D*). This positive impression will probably tear if the mold is opened too soon after the pouring.

The rough edge formed by the seam in the mold is trimmed off with a razor blade and sharp knives (Fig. 4*E*), which, if dipped into water, glide through

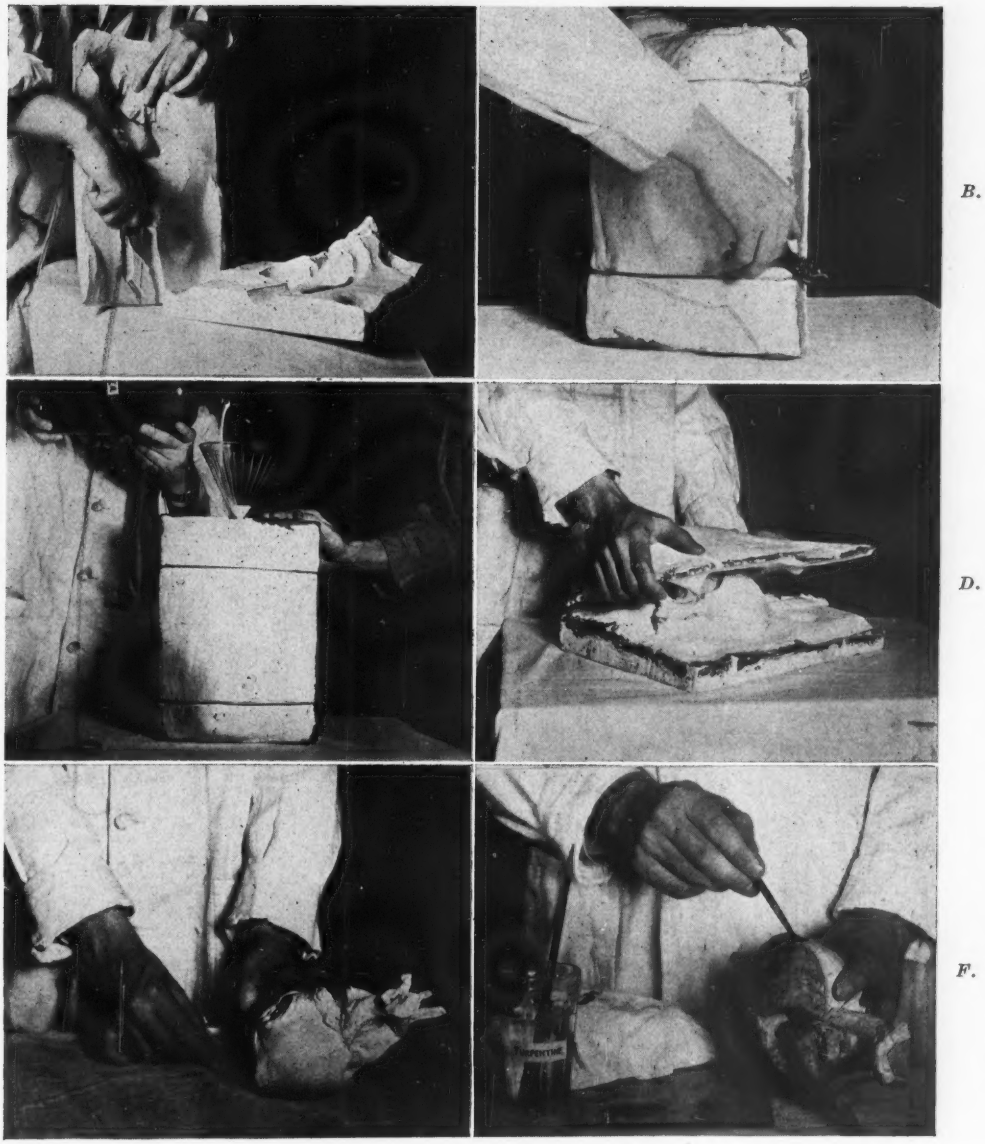
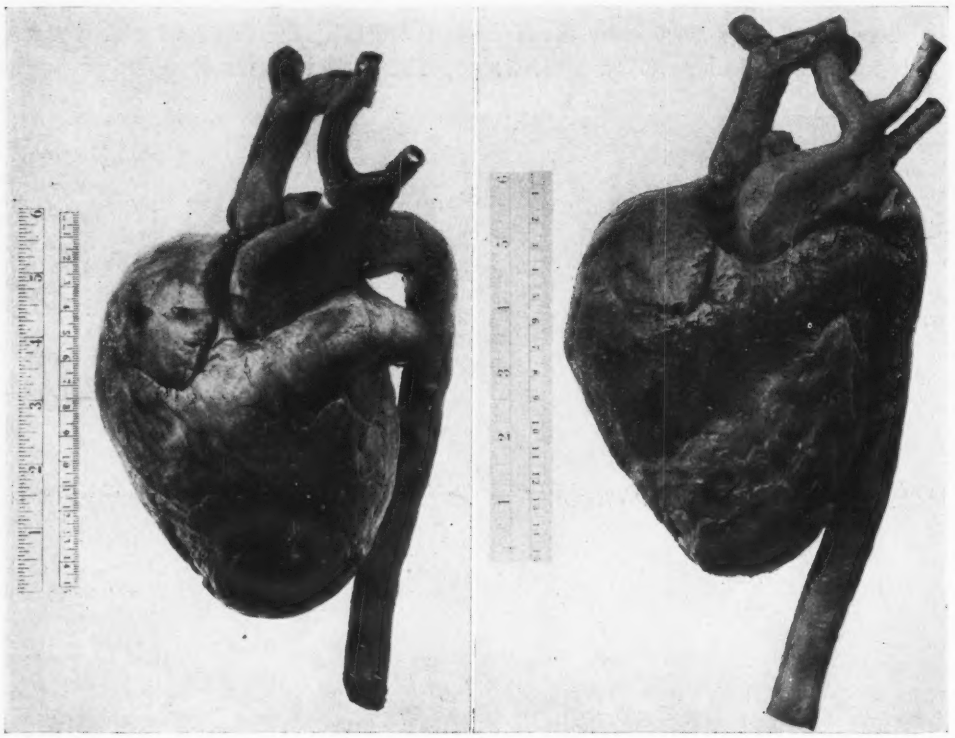


Fig. 4.—*A*, Cutting away the opening for pouring the rubber.
B, Wiring the mold together.
C, Pouring the rubber into the mold.
D, Separating the two halves of the mold.
E, Trimming away the edge formed by the line of separation.
F, Coloring the rubber cast.

the rubber more easily. Any rough areas remaining may be sandpapered smooth or worked smooth with emery wheels, wire brushes, and polishing devices, after which the specimen is given a coat of shellac.



A. *B.*

Fig. 5.—*A*, The actual preserved heart. *B*, The rubber cast of the same heart colored to resemble a fresh specimen.

Coloring is then applied to simulate either a fresh or a preserved specimen (Fig. 4*F*). This is done with transparent oil paints or tempera colors. After completely drying, the specimen is given a coat of dammar varnish to create the glossy effect of a fresh specimen. It is then mounted.

Fig. 5*A* is a photograph of the actual preserved specimen. Fig. 5*B* is the rubber cast of the specimen. The slight difference in tonal value is the result of applying more red tints to the rubber cast to obtain the appearance of a fresh specimen.

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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SCURVY, Human Experimental, Lund, C. C., and Crandon, J. H. J. A. M. A. 116: 663, 1941.

A vitamin C free diet produced scurvy in one of the authors at the end of five months, and certain symptoms of it were severe at six months.

The ascorbic acid level of the blood plasma fell to zero in forty-two days and that of the white blood cells in one hundred and twenty-two days.

A wound healed well after the patient had been three months on a scorbutic diet but not after six months.

There is no correlation between vitamin C intake and plasma levels, and the incidence of postoperative atelectasis and pneumonia.

Vitamin C deficiency may be a factor in failure of some human wounds to heal.

A dietary history should be taken on all patients who come to major surgery and, if a long-continued marked deficiency of the intake of vitamin C is found, the patient should be given from 1 to 4 Gm. of ascorbic acid daily.

AMEBA, Non-Pathogenic, Clinical Symptoms Associated With So-Called, Rothman, M. M., and Epstein, H. J. J. A. M. A. 116: 694, 1941.

Stool specimens from 406 patients located in and about Philadelphia harbored one or more of the five forms of intestinal amoebas. The most prevalent was *Endolimax nana; E. coli* was second.

These organisms, including *Diendamoeba fragilis,* had a causal relationship to their symptoms to the extent of 44.1 per cent.

The most common symptoms were constitutional; i.e., extreme tiredness and weakness. Of local nature were abdominal discomfort and diarrhea.

When a coexisting disease is active, the results to be expected may be only partial; i.e., only those symptoms for which the amoeba is responsible will disappear.

The response to carbarsone therapy is usually within three to five days. When no improvement is noted by the tenth day, one may be fairly certain that the symptoms were not due to the presence of the amoeba.

From a clinical point of view, all forms of amoeba appear to have a pathogenic role. Furthermore, since they may represent a serious public health menace and problem, it is suggested that they should be accepted and treated as such until clinical evidence to the contrary can be proved.

SULFANILAMIDES, Method for Obtaining Rapid Bacterial Growth in Cultures From Patients Under Treatment With, Janeway, C. A. J. A. M. A. 116: 941, 1941.

The authors recommend the addition of para-aminobenzoic acid to a final concentration of 5 mg. per hundred centimeters to all routine culture media. It will make cultures from patients under treatment with the sulfonamide drugs mean what they should—i.e., the presence or absence of viable organisms. By way of caution, it must be noted that the body fluids may frequently be sterilized by sulfonamide therapy and then later become positive again when the patient relapses. During the remission positive cultures could be obtained only from the local focus, and no amount of para-aminobenzoic acid would make the cultures of the body fluids positive.

PROTHROMBIN: Quick's Test Simplified by the Use of a Stable Thromboplastin, Souter, A. W., and Kark, R. Am. J. M. Sc. 200: 603, 1940.

Brains were removed from freshly-killed rabbits, and the larger superficial blood vessels were stripped off after washing with water. The brains were then ground to a paste, spread thinly on a glass plate, and thoroughly dried in an oven at 37° C. The dried residue was scraped off the plate, and about 15 c.c. of saline extract were prepared by adding 30 c.c. of 0.85 per cent sodium chloride solution to 3 Gm. of dried brain, mixing with a glass rod and incubating at 56° C. for fifteen minutes. On centrifuging the mixture at 2,000 r.p.m. for five minutes, a somewhat opalescent supernatant fluid was obtained. About 2 c.c. of this extract were set aside for testing as a control, and the remainder was placed in small serum bottles, 1 c.c. in each. The contents of these bottles were then "lyophilized," and at the completion of the process the evacuated bottles were set aside for storage at room temperature (about 20° C.).

The portion of extract which was kept as a control was tested for thromboplastic activity by using to determine the Quick prothrombin times of a series of dilutions of normal citrated plasma with prothrombin-free plasma prepared by the method of Kark and Lozner, so that the preparations contained 100, 80, 60, 40, 20, and 0 per cent prothrombin.

The technique used in the determination of these prothrombin times was essentially that of Quick. One-tenth cubic centimeter of thromboplastin was pipetted into a tube 10 by 75 mm., and the tube was placed in a constant temperature (37.8° C.) water bath. After a few minutes 0.1 c.c. of plasma was added, and immediately thereafter 0.1 c.c. of 0.277 per cent solution of calcium chloride. A stopwatch was started at the moment of delivery of the calcium chloride solution from the pipette. The tube was agitated gently in the bath for fifteen seconds and then rocked while held in the air until the contents ceased to flow freely. At this point the watch was stopped and the time was taken.

Bottles containing the dry, powdered, "lyophilized" material were opened at intervals of one, two, three, four, and ten weeks after "lyophilization," and the contents were restored to their original volume by the addition of distilled water, when a somewhat opalescent solution was readily obtained. The thromboplastic activity was then determined on each occasion by testing against plasmas containing various percentages of prothrombin. At the same time the results obtained were compared with those given by an extract freshly made up from the original dried rabbit brain substance stored in the icebox at 5° C. The figures for these results are shown in Table I.

TABLE I

QUICK PROTHROMBIN TIME OF MIXTURES OF NORMAL AND PROTHROMBIN-FREE PLASMAS, USING FRESHLY PREPARED AND LYOPHILIZED THROMBOPLASTINS

	Time in weeks after lyophilization of thromboplastin at which Quick prothrombin times were observed	Quick prothrombin times of mixtures of normal plasma and prothrombin-free plasma (seconds) Percentage of normal plasma in mixture					
		100	80	60	40	20	0
Fresh thromboplastin extract for lyophilization	Control	21	23	26	29	40	No clot
"Lyophilized" thromboplastin	1	21	24	27	32	50	No clot
Freshly prepared thromboplastin		20	21	23	28	42	No clot
"Lyophilized" thromboplastin	2	24	26	28	34	56	No clot
Freshly prepared thromboplastin		23	25	26	32	52	No clot
"Lyophilized" thromboplastin	3	23	25	28	34	54	No clot
Freshly prepared thromboplastin		21	23	25	29	45	No clot
"Lyophilized" thromboplastin	4	21	24	26	31	49	No clot
Freshly prepared thromboplastin		20	22	25	30	40	No clot
"Lyophilized" thromboplastin	10	21	23	25	33	68	No clot
Freshly prepared thromboplastin		21	23	26	33	68	No clot

STREPTOCOCCUS, Convalescent Serums (Scarlatinal), Platou, E. S., Dwan, P. F., and Hoyt, R. E. J. A. M. A. 116: 11, 1941.

The clinical and experimental evidence presented suggests the following points:

1. Erythrogenic toxin, produced in varying amounts by any of Griffith's thirty types of hemolytic streptococci, is important in the pathogenesis of scarlet fever and other streptococcic infections, and its prompt neutralization is desirable.

2. Other toxic products of the hemolytic streptococcus, such as leukocidin, fibrinolysin, and spreading and invasive factors, have received inadequate consideration.

3. The ideal antiserum for treatment of infections due to group A hemolytic streptococci (including scarlet fever) is one which is rich in both group-specific and type-specific antibodies and which is homologous.

4. Though commercial scarlet fever antitoxin has merit as a neutralizing agent against erythrogenic toxin, it lacks any antibacterial or anti-invasive properties.

5. Pooled convalescent scarlet fever serum in large doses confers considerable antitoxic immunity and in addition an effective antibacterial immunity.

6. Heterologous serum (horse) may cause reactions which can predispose to bacterial spread.

7. Attempts to increase the antitoxic titers of human convalescent donors have been unsuccessful.

8. Hyperimmunization of convalescent donors with streptococcus vaccines did not result in increased antibacterial activity under the conditions of the authors' experiment.

9. Pooling human sera according to antibody activity and type and administration of this product intravenously offer an immunologically sound basis for the serum treatment of group A streptococcus infections.

TISSUE: A Rapid Method of Staining Fat in Frozen Sections With Osmic Acid, Krajian, A. Arch. Path. 30: 766, 1940.

1. Fix tissue in a 10 per cent solution of formaldehyde for twenty-four hours.

2. Cut frozen sections 10 microns thick.

3. Bring to a boil a 1 per cent aqueous solution of osmic acid in a pyrex test tube and pour the solution into a small Stender dish.

4. Place the section in the hot osmic acid solution and keep it in a paraffin oven at 60° C. for five minutes.

5. Wash in a basin of tap water.

6. Counterstain in 1 per cent aqueous eosin or phloxin for one minute.

7. Wash rapidly in tap water.

8. Transfer the section to a slide and let drain off.

9. Place 3 large drops of glycerin jelly (previously melted in a paraffin oven or a water bath) and cover with a cover slip.

Result: The fat globules are black or gray black; the background is red.

For emergency examination, bring a 10 per cent solution of formaldehyde to boil in a pyrex test tube (60 c.c. capacity); drop a thin piece of biopsy or autopsy material into it, and place the specimen in a hot oven (60° to 65° C.) for ten minutes. Cut thin frozen sections and stain by the method described.

It is not necessary to discard the used osmic acid solution. Pour it back into the stock bottle and use it over and over.

TISSUE, Combined Stain for Fat and Elastic, French, C. Arch. Path. 30: 1243, 1940.

Method of Embedding

1. Fix tissues in solution of formaldehyde for twenty-four hours or longer.

2. Wash in running water for five hours.

3. Place in a 5 per cent aqueous solution of gelatin (Knox) at 37° C. for twelve to twenty-four hours.

4. Place in 10 per cent aqueous solution of gelatin at 37° C. for six to twelve hours.
5. Embed in 10 per cent gelatin. Harden in icebox for two to four hours.
6. Before cutting, place individual blocks in solution of formaldehyde for twelve to twenty-four hours (either blocks or sections may be preserved indefinitely in solution of formaldehyde).
7. Cut sections on freezing microtome at 10 microns.
8. Before staining, place sections in formaldehyde for twelve to twenty-four hours.

Method of Staining

1. Rinse section well in distilled water.
2. Dip several times into 70 per cent alcohol.
3. Place in fat and elastic tissue stain, warmed to not more than 45° C. for fifteen minutes.

Formula for stain:

Weigert's elastic tissue stain (ripened)	10 c.c.
Distilled water	2 c.c.
Scarlet red, saturated solution in equal parts of acetone and 70 per cent alcohol	8 c.c.

4. Rinse in 70 per cent alcohol.
5. Rinse in distilled water.
6. Place in Delafield's (or other) hematoxylin for one to two minutes.
7. Rinse in distilled water.
8. Differentiate in 0.5 per cent acid alcohol if desired.
9. Immerse in weak ammonia until the section becomes blue.
10. Rinse in distilled water.
11. Transfer to a slide, drain, and mount in glythrogel. (If desired, 1 per cent aqueous vert lumiere may be used as a counterstain after the hematoxylin, followed by mild decolorization in 60 per cent alcohol.)

DEXTROSE TOLERANCE, Oral and Intravenous, in Cases of Acute (Catarrhal) Jaundice, Pachman, D. J. Am. J. Dis. Child. 60: 1277, 1940.

The tolerance to dextrose (orally and intravenously administered), the icteric index, and the bilirubin content of the blood were determined serially in 12 children with catarrhal jaundice.

The icteric index closely paralleled the serum bilirubin content of the blood.

Total cholesterol values were normal or below normal in all but 3 patients. The percentage of esters was decreased in 75 per cent of the children.

No correlation was found between the degree of icterus and the type of dextrose tolerance curve obtained.

Decreased tolerance to orally administered dextrose was frequently found when the response to the previous intravenous test had been normal.

When the intravenous test showed a decrease in tolerance, the tolerance to orally administered dextrose was also decreased.

Improvement of a previously decreased tolerance to dextrose (orally or intravenously administered) usually accompanied clinical improvement and a decrease in the degree of icterus.

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